QUANTITATIVE ORGANIC MICROANALYSIS

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BASED ON THE METHODS OF FRITZ PREGL

FOURTH ENGLISH EDITION

Completely Revised and Edited
by
JULIUS GRANT
M.Sc., Ph.D., F.R.I.C.

WITH 94 ILLUSTRATIONS

LONDON
J. & A. CHURCHILL LTD.
104 GLOUCESTER PLACE
PORTMAN SQUARE
1945

Printed in Great Britain

PREFACE TO THE FOURTH ENGLISH EDITION

In compiling the new Edition of this standard work, it has been felt that the time has come for it to be thoroughly revised, both in scope and method of presentation, and there will be few who will disagree with the conclusion that one of the most desirable of innovations is the introduction into it of a more " international " character.

This decision can, it is believed, easily be justified. In the first place, the earlier editions were literal translations from the German, and have taken practically no account of the large volume of work on the subject published other than in German-speaking countries; this has meant the omission of many important advances. Secondly, the book was originally a description of the detailed technique of one school of microchemistry—one might almost say of one microchemist. No one will deny that this was pioneer work and masterly in its achievements, but the day has long since gone when this branch of analysis can be regarded as the monopoly of any one band of workers, however noted. Finally, much of the interval which has elapsed since the publication of the last edition has seen the almost complete exclusion of information from German sources, and until this information becomes fully available again the gap must necessarily be filled principally by work published in the English-speaking countries.

The opportunity afforded by a revision of this scale and type has been used to introduce what, it is hoped, will be regarded as further improvements. Thus the text has been condensed and clarified considerably, and each method is now presented following an orderly sequence of sub-sections, namely introduction, apparatus, reagents, procedure, calculations, notes, references. Where (as in many cases) Pregl's method is still the most reliable and convenient, this has been retained and adapted to fit into the above scheme, modifications due to other workers being introduced where appropriate.

Entirely new sections have also been included on subjects which, considered alone, might be regarded as on the borderline between organic and physical or inorganic methods of microanalysis, but which organic chemists have frequently to use. Examples are volumetric work, colorimetry, determinations of physical constants, general microchemical technique, gas analysis, etc. These have, of course, been dealt with only from the point of view of microchemical analysis, and the detail in which they are treated is roughly proportional to their importance as aids to organic analysis, as it has been considered inadvisable and unnecessary to depart too far from the scope of the book as expressed by its title. Full subject and author indexes are now provided, and numerous references are to be found at the end of each section.

These additions would have involved an appreciable increase in the size of the book, but the more concise method of presentation, the condensation referred to above, and the use of war-time standards of production have more than counterbalanced this tendency without necessitating the omission of anything of importance.

As in previous editions, the needs of the beginner in microchemical analysis have been borne prominently in mind, although a background of knowledge of ordinary organic analysis is assumed. It is hoped that the result is a book which preserves the tradition of the pioneer work on which it was originally based, but which will appeal all the more to post-war teachers, students and analysts partly by reason of its wider scope ; and partly because it is more in keeping with the inevitable and proper trend towards a world of science that is independent of national boundaries.

The author's thanks are due to Dr. Janet W. Matthews for her advice and assistance during the early stages of the production of the book.

J. G.

ACKNOWLEDGMENTS

The author gratefully acknowledges assistance in connexion with illustrations from the following :—

Messrs. A. Gallenkamp & Co. Ltd. (Figs. 7, 8, 27, 29, 35, 93, 94).
Messrs. Griffin and Tatlock Ltd. (Fig. 77).
Messrs. L. Oertling Ltd. (Fig. 2).
Professor H. V. A. Briscoe, Dr. J. W. Matthews and the Royal Institute of Chemistry (Figs. 13, 15, 16, 23, 26).

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QUANTITATIVE ORGANIC MICROANALYSIS

CHAPTER I

MICROCHEMICAL BALANCES

HISTORICAL AND GENERAL OBSERVATIONS

THE early microbalances were all designed for special purposes, such as molecular weight determinations, and consequently had a very limited load capacity. However, in order to carry out successful quantitative organic analysis on the micro-scale a microbalance capable of weighing relatively large objects of 10 gm. or more, and of varied shapes, is essential. The development of such a microbalance from its forerunner the assay balance (which had a maximum load of the order of only 2 gm.) is largely due to William Kuhlmann, whose excellent workmanship in the balance works of Paul Bunge, attracted the attention of Friedrich Emich, the pioneer of inorganic microchemistry. It was in Emich's laboratory that Pregl first saw Kuhlmann's "Assay Balance for Precious Metals" constructed in 1906, which, with a maximum load of 20 gm., weighed accurately to 10 μgm. (1 μgm. = 0·001 milligram; or, according to American usage, 1 microgm. or 1 γ).

Pregl decided that an improvement on this type of balance would be suitable for organic microanalysis and Kuhlmann, by grinding the knife edges very carefully, was able to construct for him the first microchemical balance. This was ten times as sensitive as the assay balance, and could be used to weigh to one-thousandth of a milligram. This type of balance is usually called a "microchemical balance" because the term "microbalance" has a long-standing prescriptive right as indicating the Nernst type of balance.

Microchemical balances have a wider range of use than any other balances known to-day; thus they are sensitive to differences of weight of 0·001–0·002 mgm., and objects of any shape, and of any weight up to 20 gm. can be weighed so long as they can stand, be laid or be hung on the balance pan. When the precautions recommended in this Chapter are taken an accuracy of $\pm$ 0·001 mgm. may be obtained with a weight of 20 gm.; that is, a sensitiveness of 10^{-7}. Of course, with weights of this order the accuracy of weighings will be influenced by certain unavoidable factors. Since, however, microanalysis deals almost entirely with relatively light objects, and not so much with absolute weights as with changes in weight of the order of a few milligrams as determined by difference, the stated limit of accuracy of $\pm$ 0·001 mgm. can usually be attained. It is probable that the types

of balance described below represent the limit of possible achievement in this direction.

With careful use the sensitiveness of a well-made microchemical balance is preserved for many years, and there are both Bunge and Kuhlmann balances which have been in use for over 20 years and are still in perfect order. Symptoms of ageing appear only after long and frequent use, and they are indicated chiefly by a more rapid "fatigue," but also by a fall in the sensitiveness (by, up to one-half). It is obvious that careless handling will lead to these results more quickly. It is usually only the novice who has never carried out a great deal of accurate weighing, who finds any difficulty in using a microchemical balance. Any experienced analyst can, after a little practice, use such a balance as rapidly as he can use an ordinary fine analytical balance, and without sacrifice of the requisite degree of accuracy.

CONSTRUCTION AND PRINCIPLE OF THE MICROCHEMICAL BALANCE

Kuhlmann's Michrochemical Balance

The Kuhlmann microchemical balance, sometimes called the "Pregl microbalance" (Fig. 1), has a beam 70 mm. long and will take a maximum load of 20 gm. It has a constant sensitiveness throughout

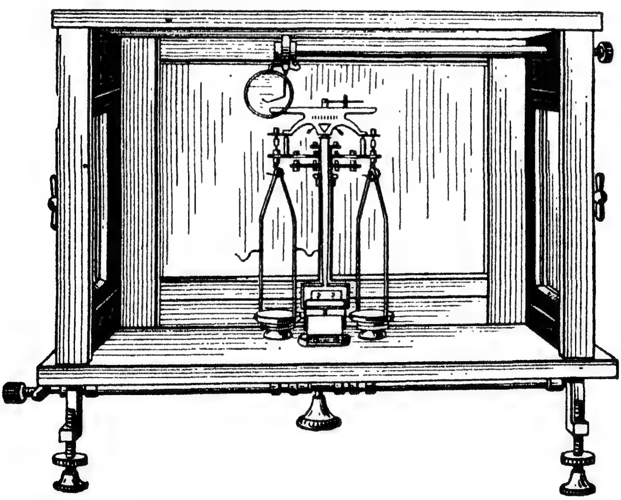

FIG. 1. Kuhlmann microchemical balance, Model 19b.

its whole range, whether loaded or unloaded, thus differing from the ordinary chemical balance the sensitiveness of which decreases with an increase in load. This constancy of the sensitiveness is due to the facts that the three knife edges are so accurately ground that they are optically straight; that they are adjusted so as to be parallel to one another and in one plane; and that the beam is rigid and undergoes

no detectable distortion even with the maximum load. The balance has a rider beam carrying a lens and a rider holder, so that the rider (which weighs 5 mgm.) can be moved from outside the balance case. All the suspension points are of agate, and there are supports of agate under the balance pans which operate when the latter are at rest. A small screw is provided for zero-point adjustment (see p. 10).

The balance is housed in a small glass case, with a counterpoised sliding front and side doors. The beam release is operated by a small handle which can be fitted either under the left or right side of the balance; the former is preferable, since the right hand is then free for adjusting the rider. The arms of the left-hand pan are provided with hooks for supporting apparatus. The balance is so constructed that it is in equilibrium when unloaded and when the 5-mgm. rider is in the first notch on the left. A displacement of the rider to the one hundredth notch (the last on the right) corresponds with a load of 10 mgm. on the right pan; the numerals stamped on the beam below each tenth notch thus indicate whole milligrams. Correspondingly, a displacement of the rider by only one notch to the right corresponds with an increase in load of 0·1 mgm. on the right pan of the balance, and this produces an alteration of the equilibrium position of ten divisions on the pointer scale; thus, one division of the pointer scale corresponds with 0·01 mgm., and as with very little practice, the oscillations of the swinging pointer can be read to one-tenth of a division, it follows that, if all the necessary conditions are observed, an accuracy of $\pm$ 0·001 mgm. (*i.e.*, $\pm$ 1 γ) is obtainable by a worker having normal eyesight. Difficulties in reading occur with observers with abnormal eyesight, and particularly with those who are astigmatic or seriously myopic, especially if these defects are not properly corrected by spectacles (see p. 8).

Oertling's Microchemical Balance

The pointer swings of this balance (Fig. 2) are easy to read by reason of a prismatic reflecting device, somewhat similar to that of the Bunge model (p. 4), and by means of which the image of an accurately divided graticule on the end of the pointer is projected on to a conveniently placed screen at the top of the balance case. The scale is divided into 200 consecutively numbered divisions, the central marking being 100, so that the reading can be added to or subtracted from the rider reading, according as the object is heavier or lighter than the weight read from the rider. The sensitiveness is thus 0·001 mgm. per half-division. To read such a scale involves no strain on the eyes, but the balance must, of course, be housed in a dark place for the lamp and lens projection system to be effective; the system also suffers from the general disadvantage of a telescope method in that the whole pointer-scale cannot be seen at once, thus lengthening the weighing time. On the other hand, the use of a special glass for the case reduces any heating effects due to the projector lamp.

Other special features of the balance are the 5-in. nichrome beam,

the vertical type rider-slide graduated from 1 to 10 in 100 divisions; the specially shaped 5-mgm. rider; and the special steadying-pins on the pan supports, which minimise oscillation when the balance is first brought into operation. The influence of eddy currents is largely

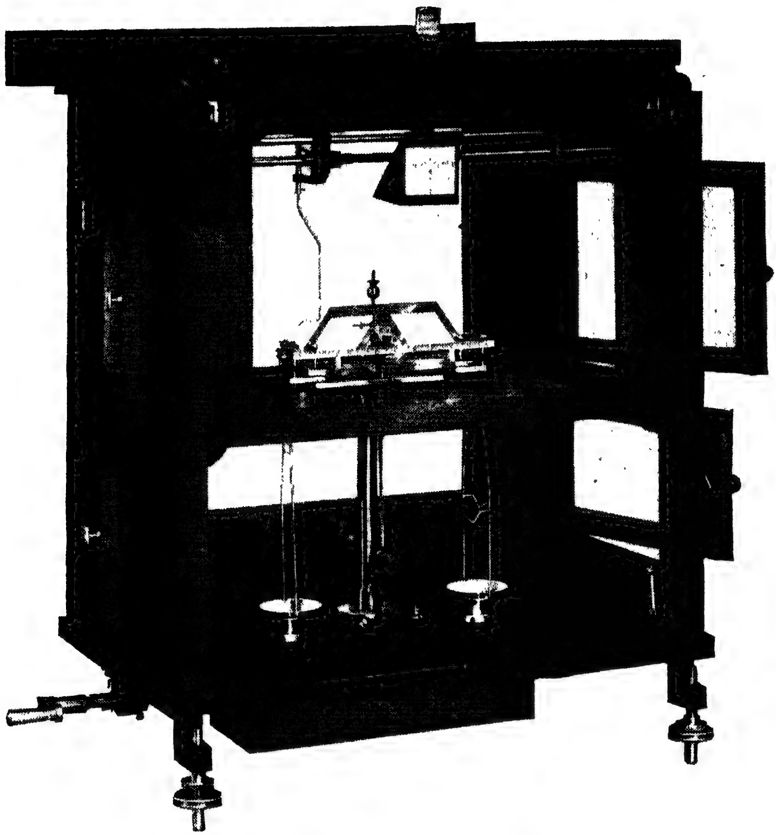

FIG. 2. Oertling microchemical balance.

eliminated by a glass partition which shuts off the upper from the lower portion of the balance. Both portions are, however, accessible by side-doors; or for cleaning and adjustment, by removing the whole of the glass case, which is hinged at the back.

P. Bunge's Microchemical Balance

This, like the Kuhlmann balance, is developed from the first assay balance and is constructed on the same principles. Only differences and improvements therefore, will be considered.

Since one of the Bunge models is arranged for a maximum load of 30 gm., the beam of this balance is 140 mm. long; thus the oscillations are slower. On the other hand, the distance between individual notches on the rider beam is twice as great as on the Kuhlmann balance, so that a lens for the rider is no longer necessary and, according to observations

made by Schwarz-Bergkampf,[1] smaller errors result from placing the rider inaccurately. The shape of the rider (Fig. 3) and the deeper cutting of the notches also facilitates the correct placing of the rider (*cf.* p. 8).

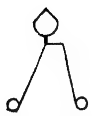

FIG. 3. Bunge rider.

The Bunge method of protecting the suspensions and beam shown in Fig. 4 is particularly useful for balances to be used by students. It prevents the beam from slipping on its point of support owing to the dropping of the suspensions; this may happen following collisions with absorption apparatus being weighed.

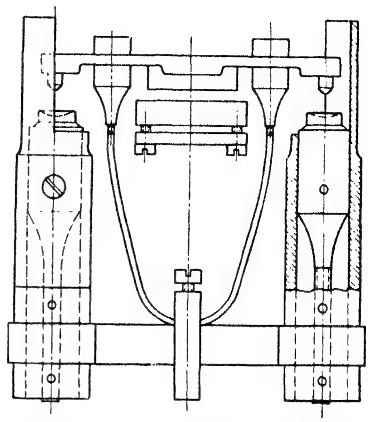

FIG. 4. Bunge method of protection for suspension and beam.

The two large doors in the model shown in Fig. 5 compel the right hand to be used for the right balance pan and the left hand for the left pan. The rider scale and the suspensions are readily seen through the fixed front glass window. Since 0·001 mgm. can easily be estimated at a distance of 50–60 cm. in front of the balance; and since, moreover, the macro-scale at the

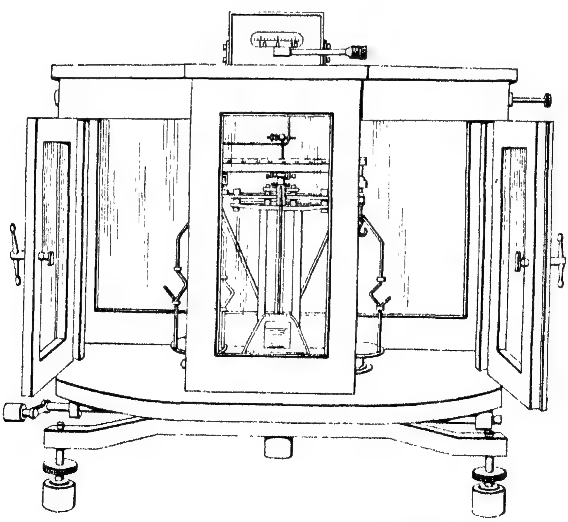

FIG. 5. Bunge microchemical balance.

foot of the pillar (for rough weighing with the rider) can be observed from this distance by reflection in two mirrors, it is unnecessary to approach the balance too closely when the arrestment is liberated. The influence of the warmth of the body is thus practically excluded.

Aperiodic Balances

In balances of this type the rate of weighing is accelerated by damping down the oscillations so that the balance comes to rest without swinging; the displacement of the pointer from zero, as read directly from the pointer scale, then gives the last decimal place. The damping device is usually a pair of concentric hollow cylinders of slightly different diameters, one pair at each end of the beam. One cylinder of the pair is fixed to the column of the balance, whilst the other is fixed to the top of the pan support and moves inside the first with the motion of the beam; the resistance to compression or expansion of the air between the end of the cylinders thus serves as the damping medium. Air is preferable to oil or magnetic methods for this purpose.

In a well-made balance of this type such a damping arrangement also greatly minimises the effects of vibration; thus, Furber [2] records that tramcars only 20 yds. away from the place where such a balance was mounted were without effect on it, whilst other microchemical balances soon became unusable. With the Kuhlmann model, it is very important that the balance should always be correctly levelled, as otherwise the cylinders may touch and braking occurs too readily. The bottom back wall, and both side walls of the case of the Bunge balance are made of aluminium, for thermal and electrostatic equalisation; this, however, is of little advantage, because although the metal walls equalise differences of temperature inside the balance, they are still subject to the temperature changes of the balance room.

In the Kuhlmann model equilibrium to the nearest 0·1 mgm. is obtained by first using the weights and then the 5-mgm. rider. Readings to 0·01 and 0·001 mgm. are then made directly, with a telescopic lens, on the scale which extends from left to right and is divided into 100 parts; one division represents 0·001 mgm. The braking device provided is very easily applied and withdrawn, and by its means the rapidly oscillating balance is so retarded that the position of equilibrium is very quickly attained. Its essential feature is a small, short beam, carrying an excess weight of 1 mgm. By means of this a flat-ground agate plate is pressed against a highly polished rotary member, which is attached to the balance beam in such a way that its axis is in line with the middle knife-edge. The telescope is central above the balance case, and by the use of two prisms a high magnification of the pointer reflections is attained; it is, therefore, very important that the balance supports should be free from vibration.

In the actual weighing operation equilibrium is first restored to within 0·1 mgm. in the usual way by adding weights and adjusting the 5-mgm. rider. The balance is then arrested, the lever-brake applied by liberating the suspended lateral weight and then the arrestment is again liberated. The indicator is then seen through the telescope to move into the new position of equilibrium, and to come

to rest there or in its vicinity. When the lateral weight is then lifted and the brake thus removed, the indicator moves further through a few divisions, and finally comes to rest after the brake has been repeatedly applied and removed.

It is apparent that the manipulation of this balance requires a little practice. The balance is, however, particularly convenient for operations such as weighing substances into boats, weighing absorption tubes and halogen filter tubes, etc., as by observing the travel of the image through the telescope it is easy to ascertain when temperature equilibrium between the object and the air in the balance case has been attained. Unfortunately, the anticipation that this balance might enable the accuracy of weighing to be multiplied tenfold, as compared with those previously described, has not been realised. In spite, therefore, of the more obvious advantages of observing the position of a stationary line of light instead of the turning points of a rapidly swinging pointer, the latter method continues to be preferred.

The Ultra-microchemical Balance

Normally it is not necessary to weigh to a greater degree of accuracy than $\pm$ 1 μgm. In some cases, however (*e.g.*, when working with 1 mgm. of material), there is need for an instrument which is ten times more accurate, *i.e.*, the thousandth part of a milligram is read, not estimated, and only ten-thousandths are estimated.

According to Schwarz-Bergkampf,[1] the differences in the intervals between the separate notches, which are measured in a horizontal microscope, amount to 0·006 mm. Thus, at the worst, for a distance of 0·7 mm. between the notches, the error in weighing is 1%. With the usual microchemical balances this variation falls within the experimental error, but it would affect results on an ultra-microchemical balance to the extent of 10%. If, on the other hand, instead of the 5-mgm. rider, a 0·5-mgm. rider is used for constant intervals between the notches, then the error of position (while still 1% with respect to the 0·5-mgm. rider) is only 0·0001 mgm., *i.e.*, 0·1 μgm. By the use in this way of a Kuhlmann ultra-microchemical balance with two rider scales it is possible to weigh less than 1 mgm., with an accuracy of 0·25 μgm.

In addition to the usual types of equal-armed balances, there are also other highly sensitive instruments, which, however, by reason of their small loading capacity, cannot be used if the objects to be weighed are relatively heavy in comparison with the amount of material used. Wiesenberger [3] has also been able to improve Knut Ångström's electrometric compensation balance to a high degree, and to carry out determinations of residues in small platinum dishes and microelectrolyses (p. 123) with great accuracy.

Other types of balances have also been suggested for microchemical work, *e.g.*, those operating on electromagnetic or vacuum principles; they are, however, not in common use. Further information is

obtainable from a paper by Gorbach,[4] which also contains a full bibliography.

CHOOSING A MICROCHEMICAL BALANCE

Most balance makers of repute now market a microchemical balance of the Kuhlmann type. They are all somewhat similar in general construction, but may differ in workmanship, so that when purchasing such a balance it is advisable to test it thoroughly beforehand. The principal points to be watched are indicated below.

The rider notches on the beam should be carefully examined with a lens for any irregularities, and they should have the form of a rather acute V so that the rider readily falls to its correct vertical position in the pointed base. The balance should work smoothly, and every part should fit easily into its proper position. It is essential that the release should be very well adjusted, so that the pointer swings through the correct distance for reading the scale. If the purchaser has not a very wide experience of microchemical balances it is advisable for him to compare the details of workmanship with those of a well-established model, such as a Bunge or Kuhlmann. The sensitiveness should be also tested over the whole range of loads, say 0, 1, 3, 5, 7, 10, 15, 20 gm., and a series of weighings of the same object should be made at 5-min. intervals for about 30 min. If the result is satisfactory the sensitiveness is the same for the different loads, and the same object will weigh the same to within $\pm$ 0·001 mgm. If possible the procedure indicated on p. 10 should be applied, and all such tests should, of course, be made by an experienced microchemist.

The microchemical balance is used to weigh to 0·001 mgm. The milligrams and tenths of milligrams are read by means of the rider as with an ordinary chemical balance, and the hundredths and thousandths of milligrams from the swinging of the pointer. It is therefore essential that the pointer scale, which is extremely small, should be readily visible, so that the swings can be read without eyestrain. Several devices are available for magnifying the pointer scale ; *e.g.*, a telescope lens fixed on the front of the balance case, or an ordinary lens inside the case. Originally a concave mirror was used, the scale being fixed with its back to the observer, who only saw its reflection in the mirror ; the pointer had a large rectangular hole in it, so that an uninterrupted view of the mirror was obtainable. The choice is entirely a matter for the individual to decide. Workers with weak eyesight will probably prefer the telescope model, which is easier to use, although it probably strains the eye more. Its principal disadvantage is that the whole pointer scale cannot be seen at one time. In general, for teaching purposes a mirror type is recommended, but for research work or for use by one or two selected workers it is better for those concerned to select the model that best suits their eyesight. This is an important point, quite apart from any question of ease of working, because astigmatic or seriously myopic workers tend to bring their heads too

near to the balance, and their breathing and the heat of the body may influence the regularity of the oscillations of the pointer.

THE ERECTION OF THE BALANCE AND SOURCES OF ERROR

Firstly, it is very important for the satisfactory working of each balance that the directions for mounting, which are sent with it, should be followed most accurately, as otherwise it is easy for the enthusiastic beginner to cause serious damage. Suitable support for the balance is particularly important. Marble slabs 3–4 cm. thick, supported on iron bearers which are mounted in the wall, are used with sheets of lead between the marble slabs and the iron bearers. The feet of the balance are inserted in vibration absorbers with rubber discs, which are often supplied with the balance.

Vibrations from machines or centrifuges may pass through walls, especially of concrete buildings, and may be transmitted through the iron bearers to the balance. Such disturbances are completely avoided by the use of a balance table, which stands away from the wall with its legs supported on two heavy concrete pillars. Between the floor and the bases of the pillars are placed several sheets of lead, 1·5–2 mm. thick. In this way balances are completely protected from vibrations of every kind. Frequently sheets of cork or rubber are recommended instead of lead as an insulating material. However, instead of a vibration-absorbing table-top insensitive to pressure, we then have one which reacts to any chance pressure such as one-sided loading (*e.g.*, due to desiccators) and the zero of the balance may be altered.

In choosing a place for the erection of the balance, the lighting and heating arrangements of the room are, however, often more important than freedom from vibration. Whilst a good microchemical balance is little affected, if at all, by the passage of a tramcar in the vicinity (see p. 6), it is very sensitive to air currents within the balance case. Therefore it is inadvisable to mount the balance in front of a wall in which there is a heating unit, or near a stove ; it is best placed against the opposite wall. The balance must never be exposed to direct sunshine, and the presence of a lighting unit in the immediate neighbourhood should be avoided. Incidentally, when an illuminated pointer scale is used, the source of light for it should be switched on only when a reading is being made. Such influences cause deflections of the zero-point which, if they are small and tend to a constant value (such as those due to the change from daylight to artificial light), do not affect the accuracy of the weighings so far as we know. Frosted electric lamps, not less than 90 cm. above the top of the table and immediately over the balance, are most suitable for the artificial lighting of balances. If it is not possible to find a suitable place for the erection of the balance, any variations of the zero-point must be taken into account, particularly in the case of drying operations, which may last for hours or days.

Even in balance rooms in which all the above requirements have

been taken into account, some alteration in the zero occurs during the course of a day's work (see Furber [2]). The position of the zero of all microchemical balances depends on the temperature. Since it is very seldom possible to keep the temperature of the balance-room constant to $\pm$ 0·5° C., a determination of the zero-point must be made for all weighings which are spread over more than an hour, as follows :—

1. The zero-point deflection is expressed by the amount (in thousandths of a milligram) by which the unloaded balance varies in successive readings made at intervals of time. It is considered to be positive if the alteration is from the left to the right, and negative if in the reverse direction.

2. The true weight is then found by adding to the observed weight the zero-deflection with the reversed sign ; thus, a positive zero-point deflection must be subtracted from the weight, and a negative value must be added to it.

Schwarz-Bergkampf,[1] with Emich, investigated the precautions necessary on weighing, and was thereby able to make valuable additional determinations of the errors which arise if the temperature of the body weighed differs from that of the balance room ; or if two weighings separated by a time interval are carried out at different room temperatures. According to these observations, a balance room temperature as constant as possible, with daily fluctuations of less than 1° C., ensures a constant zero. These determinations confirm the standard conditions for weighing which have been established for 20 years.

One may readily demonstrate the effect of air currents inside the balance case as follows :—Place a body at a temperature only slightly higher or lower than that of the surroundings (for instance, a block of copper) near the left pan of the balance ; after a short interval, there is deflection to the left or to the right, respectively. The zero-point returns to normal after removing the disturbing object. On touching the side door of the closed balance with the palm of the hand a zero-point deflection is also observed. On then opening the balance front and both side doors the zero-point is restored. It follows that during weighing no objects (weight forceps, copper block, etc.) should be put in the balance case ; and, on the other hand, that objects which are always necessarily used when weighing (weights, counterpoises, counterpoised flasks, together with the necessary supply of shot for the latter), should be kept permanently in the balance case, where, moreover, they are best protected from dust.

Particularly dry conditions may give rise to errors due to the effects of static electricity. The humidity of air needs to be below 50 per cent. for these to occur, and the action of a high-frequency spark discharge coil has been suggested for their dispersal.[5]

WEIGHTS

Since the general introduction of weighing with a counterpoise in 1912 (see below) only a few of the weights which are normally provided

with the microchemical balance are used, namely, the 1-gm., 0·5-gm., 50-mgm., two 20-mgm., and the 10-mgm. weights. The accuracy of these must occasionally be tested, as after use for some years they show a remarkable tendency to become heavier. By brushing them, washing them with water and alcohol, and finally polishing them carefully with a cloth, or in extreme cases with a coarse wrapping paper, they may be restored to their original exact correspondence with the rider readings. The rider, if made of thin platinum wire, also becomes heavier in the course of time and sometimes strongly discoloured; the small weights then appear to be too light. Such riders can be restored so as to be equal to the 10-mgm. weight by careful washing in dilute potassium cyanide solution, followed by gentle friction between the finger-tips. Riders made of aluminium wire, however, are larger in volume, do not become discoloured and remain, in general, in good agreement with the other weights; and gold riders especially, remain constant in weight for years. The slight increases in weight appearing later are easily reduced, as described above. Decreases in weight seldom occur; in such cases a new rider is obtained and its accuracy is tested against the set of weights.

For this test the zero of the balance is first determined with the rider in notch 0. The 10-mgm. weight is then placed on the left balance pan and the rider in notch 100. While the rider is in the same notch the 10-mgm. weight is placed on the right balance pan, and the two 20-mgm. weights are checked successively on the left pan. Then the 50-mg. weight is placed on the left pan and tested against both 20-mgm. weights on the right pan, the rider being in notch 100.

The weights are best kept in the balance case in a small flat dish, the bottom of which is lined with black velvet. For reasons already indicated the hand should not be in the neighbourhood of the balance for longer than is absolutely necessary for operating the arrestment, and it should be the rule to leave the balance case open before beginning a series of weighings in order to ensure complete elimination of differences of temperature and moisture content between the air inside and outside the case (" acclimatisation ").

SPECIAL WEIGHING PRECAUTIONS AND ASSESSMENT OF ERRORS

The weighing of specific objects is dealt with in Chapter II, and elsewhere in this book under the appropriate headings.

It is advisable to count deflections by taking the midpoint of the scale as zero and the tenth part of a division as unity; for instance, a deflection of 2·7 scale divisions to the right is described as " 27 right," and a following deflection of 3·4 scale divisions to the left as " 34 left." The mean deflection from the zero point in this case is " 7 left," that is, one must subtract 0·007 mgm. from the sum of the weights on the right-hand pan and the weight corresponding with the

rider reading. If the readings were "39 right" and "30 left," then 0·009 mgm. would be added to the above sum.

The sensitiveness of the balance, however, is not high for readings of 100; also more fatigue is caused by large than by small deflections. It is therefore a rule not to allow the pointer to swing more than six scale divisions, and never to read differences larger than 50 units. For larger differences, the rider is placed in the next notch. If the pointer swings too far, it is advisable to stop it as it swings through the zero, and to bring it into motion again by releasing the arrestment more slowly.

It is also advisable to note down the deflections in writing. Mental calculation is distracting, and many workers tend also to find the same differences when estimating successive swings subjectively. Since the differences between separate whole oscillations cause only very small and regular reductions in the swing, comparison of the swing differences on paper is more objective than is mental calculation. This method of observing the swings enables the accuracy of the weighing to be tested at any time. Thus a deflection to the right side of the scale is added to the weight, and that to the left must be subtracted from it. If therefore the rider is displaced after a positive deflection has been obtained, by one notch to the right, the deflection will become negative and to the left. This, when added to the former positive deflection, should result in a value of 100, if 100 is the sensitiveness of the balance. In this manner the correct adjustment of the sensitiveness of the balance, both unloaded and on maximum load, can be checked easily.

It should be pointed out at this stage that although figures are often given in the literature for the sensitiveness of a balance, they are not always founded on actual scientific measurements of this quantity, but more often on the general impressions of the user. The work of Corner and Hunter [6] on the measurement of the performance of a microchemical balance is therefore very pertinent in this connexion. They find that the probable error of a single weighing, *i.e.*, $0{\cdot}6745 \sqrt{\text{mean square deviation from the mean}}$, is very suitable for this purpose, since it indicates the degree of scatter of repetitions of the same weighing. Thus, if the probable error of a single weighing is found to be $\pm$ 0·00x mgm., this means that there is an even chance that one weighing in 1, 3, 5, 20 and 100 weighings will have an error of at least 0·00x, $1{\cdot}5 \times 0{\cdot}00x$, $2{\cdot}0 \times 0{\cdot}00x$, $3{\cdot}0 \times 0{\cdot}00x$ and $3{\cdot}75 \times 0{\cdot}00x$ mgm., respectively.

The method was actually applied to eight microchemical balances by repeatedly weighing one 1-gm. weight against another. Six of these balances had mean probable errors for a single weighing of $\pm$ 0·003 mgm., which means that the error contributed to an analytical result from this source only is not likely to exceed 1% more than once in 1,000 analyses; but that it may exceed 0·5 and 0·1% once in 20 and in two out of three analyses, respectively. It is interesting to note that the two other balances had been used by students, and that their precision was only about one-third of the mean precision of the others.

A Committee of the American Chemical Society [7] which has studied this same problem also explodes any false impressions that may be derived from the fact that a balance will respond to a difference in weight of 0·001 mgm. Each participant in the study, which covered 29 balances, was asked to weigh two 1-gm. weights, 10 times, one against the other, each weighing being followed by a zero-point determination; the same procedure was also applied to two 10-gm. weights. The standard deviations were then calculated from the formula $\sqrt{(\Sigma d^2/8 \cdot 5)}$, where d is the deviation (in 8 μgm.) of each of the 10 readings from their mean. The median value of the standard deviations found was $\pm$ 3·4 μgm., the probable error being 2·3 μgm., a result in good agreement with that obtained by Corner and Hunter. Only about one-third of the reported figures were such as would allow 5-mgm. samples to be weighed to within 1 : 1,000, and it is suggested that a test of the above nature may well prove useful to the analyst as a guide to the true practical performance of his balance.

It is useful also to note the studies of Corner and Hunter on the possible sources of the errors observed. They first determined how the calculated point of rest of the swinging balance depends on the number of readings made and on the amplitude of swing, and they also compared successive weighings of the same counterpoised object without adjusting the balance between readings. The error involved in placing the rider on the beam was then taken as the residual error, *i.e.*, it was obtained from the difference between the total error and the errors due to the other above causes. The results confirmed the conclusions of Kuck and Lowenstein [8] that the uncertainty of placing a flimsy rider in a comparatively coarsely cut notch produced the major error, and this may well be the case with most microchemical balances, since a 5-mgm. rider has only to be about 0·008 mm. out of position in order to alter the weighing by 0·001 mg. on an 8-cm. balance beam. Such errors are reduced, though not eliminated, by the use of quartz rod riders.

Counterpoises

It is advisable to prepare suitable counterpoises for weighing objects which are continually in use (*e.g.*, boats for the carbon determination, platinum crucibles, Neubauer crucibles, filter tubes, absorption apparatus). Such counterpoises have long been used in technical laboratories. Thus a counterpoise (Fig. 6, *a*) for the platinum boat is made from aluminium wire 2 mm. thick, which is bent twice so that its three portions correspond with the three edges of a tetrahedron. This counterpoise is adjusted by filing until it balances the boat to within about 1 mgm. Hence, in weighing the boat the rider alone is used, without additional weights. To save time in finding the appropriate counterpoise, the counterpoise is stamped with the number of the small desiccator in which the boat is always kept. Small counterpoises of fine aluminium wire weighing about 5 mgm. are very convenient, as they enable one quickly to adjust the position of the rider.

For counterpoising heavier objects (absorption apparatus and filter tubes) thin-walled glass flasks (Fig. 6, *b*), which can be obtained marked with consecutive figures, are used. A special counterpoise flask may be prepared for each piece of apparatus with the help of the necessary amount of small lead shot (size No. 15). It is advisable in preparing such a counterpoise, first to load the flask on the right balance pan with coarse shot and to place beside it a 50- or 100-mgm. weight. As soon as the balance swings to the left replace the weight by finer shot until the balance again swings to the left; then remove one shot and ascertain whether the rider must be brought beyond the number 5. If this is the case, then still finer shot is placed on the right pan; if this result is satisfactory, the shot is placed in the flask. This is the quickest way of effecting the adjustment, and the small amount of work necessitated by preparing these counterpoises is amply repaid during subsequent weighings.

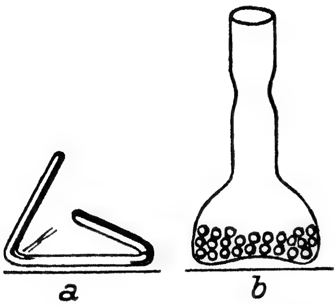

FIG. 6. (*a*) Aluminium counterpoise. (*b*) Counterpoise flask with shot. Actual sizes.

In recent years weighing tubes with long or short glass handles have come into general use, in place of tubes with aluminium handles. They are used for all weighings except those in boats and capillaries. The glass weighing-tubes (Figs. 9, 10, and 45) are so made that their weights only differ from 0·5 gm. or 1 gm. by 0·1 mgm. The rider alone is then necessary for weighing, and space is saved in the balance, because the 500-mgm. or 1-gm. weight from the set is used as a counterpoise.

Cleaning the Balance

It is necessary to clean the balance thoroughly from time to time, particularly when the arresting contacts adhere and the pointer or beam is caught on one side on releasing the arrestment. Careless erection of the newly delivered balance may cause this to occur for a considerable time.

The doors are opened and, in this order, the rider carrier, pans, suspensions, and beams are removed. The last-named parts are laid in order on the rider carrier, the floor-plate is well cleaned with moist gauze, and the balance pans and suspensions are rubbed with chamois leather which has been washed free from fat (with ether) and acid (with water) and well dried. The notched rider scale of the beam is carefully brushed with marten hair brushes, and all arresting contacts (on beam, suspensions and pillar arrestments) are finally rubbed thoroughly with a dry chamois leather. In one case of particularly obstinate stickiness Pregl finally achieved success by painting the pans and the hemispherical arrestments with a paste of freshly ignited talc and alcohol, drying, and rubbing off the talc with chamois leather.

Finally, the knife-edges and the corresponding supports are also cleaned with dry chamois leather. This operation is aided by the use of a watch-maker's lens, which is indispensable both for mounting the balance and for other work, and should always be found in the pocket of the analyst. The balance is now re-erected and tested to see whether the zero-point has altered, as is usually the case. If the zero-point deflection is large, it is corrected approximately by means of a small screw on the vane, but the handling of the screw with the fingers warms it and causes an additional deflection of the zero-point which lasts for some time. It is therefore particularly desirable, when correcting small variations, to turn the screw with the forceps in order to avoid this effect. Some manufacturers have provided a vane with light wings instead of the screw; this vane can readily be turned with the open forceps. Bunge balances have a device which permits the wing-screw to be moved from the outside by means of a forked rod. The final adjustment of the zero-point, which is concerned with the last 0·01–0·02 mgm., is best effected by means of the two levelling screws on the case.

Very fine hairs under the central knife-edge, under the suspension knife-edges, or on the end of the pointer, may obstruct the oscillations and prove very troublesome. Such faults are liable to pass undetected by the novice unless attention is directed to them.

Although the zero-point of the balance is usually altered by dismantling and cleaning, the sensitiveness need never be affected by these operations so long as they are carried out with reasonable care. The two vertically adjusted counter-screws which control the sensitiveness are screwed so tightly that their positions cannot be changed by ordinary handling, since on the suggestion of Pregl the vane for the zero-adjustment is not attached to the beam between these two counter-screws, but is independent of them. One must, however, distinguish between temporary alterations in sensitiveness, due to warming the vertical screws with the fingers, and permanent alterations which are due only to misuse.

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CHAPTER II

GENERAL MICROANALYTICAL TECHNIQUE

In this chapter details will be given briefly of the general technique used in the auxiliary operations of quantitative organic microanalysis. The determination of physical constants is dealt with in Chapter V.

WEIGHING METHODS

These notes have special reference to the weighing out of definite weights of samples for analyses (*e.g.*, for the determinations of the elements), and they therefore supplement those given in Chapter I.

Solids are weighed in boats of platinum, porcelain or resistance-glass. It is advisable to weigh substances containing arsenic, mercury or compounds of alkali or alkaline earths which have to be mixed with potassium dichromate, in boats of porcelain or of resistance-glass. The boats are boiled with 50% nitric acid before each analysis, ignited on a platinum hook in a non-luminous flame, and then placed on a copper block (Fig. 7) in a small desiccator (Fig. 8).

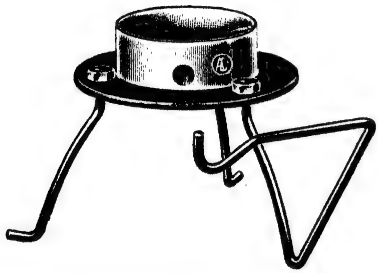

Fig. 7. Copper block.

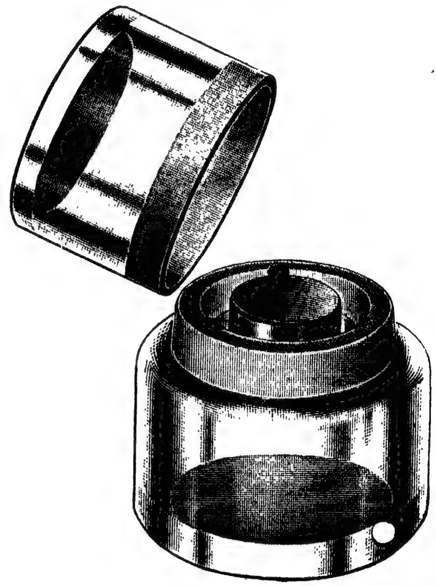

Fig. 8. Micro-desiccator containing copper block.

The high heat-conductivity of the copper block enables the boat to cool rapidly to room temperature ; weighing may begin after a few seconds for a platinum boat, or after 10 mins. for porcelain or glass boats. The boat is transferred to the left-hand balance pan by means of a platinum-tipped forceps, the aluminium counterpoise is placed on the right-hand pan and the boat is weighed accurately to 0·001 mgm. It is then placed on a clean tile and filled with 2–5 mgm. of the substance to be analysed, with a microspatula. Larger quantities (*e.g.*, 5–7 mgm.) are necessary only if very small proportions of the constituents to be determined are present. Before the boat is replaced on the balance it is held with the forceps and its sides and bottom are

brushed with a dry, dust-free sable-hair brush, to remove particles sticking to the outside. When the weighing has been made the boat is replaced on the copper block in the desiccator.

Very Volatile Solids are pushed into a capillary closed at one end, which is afterwards sealed, as in Rast's micro-determination of molecular weight (p. 198). Thus, a thin-walled capillary (diameter, 2·0–2·5 mm.) is prepared from a test-tube in a non-luminous Bunsen flame, sealed at about 40 mm. from its mouth and drawn out to a solid handle about 15 mm. long (Fig. 9, *a*). The capillary is then placed on the copper block in the desiccator and weighed to 0·001 mgm. after a few minutes ; 3–4 mgm. of the substance to be analysed are removed from a watch-glass by means of a second, longer capillary tube, open at both ends, which exactly fits into the bore of the weighed capillary. The substance is pressed in with a spatula, and the residue adhering to the outside is carefully brushed off with a marten-hair brush. The

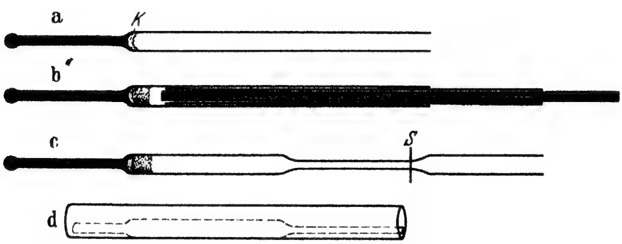

FIG. 9. Weighing capillaries for volatile solids. Actual sizes. (*a*) Capillary with solid handle containing a fused crystal of potassium chlorate (*K*). (*b*) Inserting the sample. (*c*) Sealing the capillary (at *S*) after weighing. (*d*) Platinum cylinder containing a capillary.

weighing capillary is placed, with the handle underneath, in a short glass tube, 25–30 mm. high, which is sealed at the bottom and is held vertically in a cork stopper. The capillary containing the substance is now inserted to within 5–10 mm. of the bottom (Fig. 9, *b*) and the substance is pushed out of it into the weighing capillary, by means of a thin glass rod ; the glass rod is first removed, and then the capillary. The weighing capillary is softened in the flame of the micro-burner at about 20 mm. above the substance, and is drawn out to a finer capillary (*S*) in the outer part of the flame (Fig. 9, *c*), and weighed when cool. It is advisable that *S* should not be narrower than 0·5 mm., because it may become blocked by recrystallisation of substances of high melting-point.

Viscous Substances (*e.g.*, oils of low vapour pressure) are transferred to a boat with a very thin glass rod, care being taken that the outside of the boat is not wetted.

Liquids of Low Vapour Pressure. Capillaries (length about 100 mm., diameter 1 mm.) are melted in the middle (Fig. 10) to a drop, which is drawn out to a glass rod about 25 mm. long (*b*) and sealed through in the middle (*c*) ; the ends of both handles are rounded off to small spheres. A crystal of potassium chlorate is then placed at the sealed end of such a capillary, and is fixed there by melting (*d*). At about

20 mm. from the closed end the capillary is softened, drawn out to a hair-capillary (diameter not less than 0·1 mm.) in the outer flame, and broken off at 25–30 mm. (*d*).

After the capillary is weighed it is held in forceps and the air removed by careful warming, or by exhaustion (see below). The open tip is at once inserted in the liquid, 3–5 mgm. of which are drawn in. To remove the traces of liquid remaining on the inner walls of the fine point (which would be lost on opening the hair capillary), the empty portions of the capillary are drawn quickly through the flame two or three times; the fine end is thus sealed (*e* and *f*), and the tube is weighed after a few minutes.

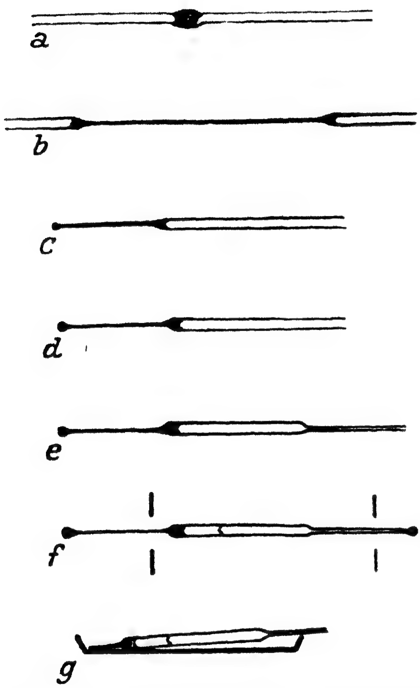

FIG. 10. Weighing capillaries for liquids. Actual sizes. (*a*), (*b*), (*c*) Preparing the capillary. (*d*) Capillary containing fused potassium chlorate. (*e*) Capillary with open end drawn out (first weighing). (*f*) Sealed capillary, containing sample. (*g*) Capillary with handle removed and point broken off, in platinum boat.

A useful method which involves exhaustion, instead of heating, is described by Alber.[1] The weighed capillary is held in an inverted position over a micro-centrifuge cone which contains the sample (and if desired a drying agent, though the top of the tube must not reach the latter). The cone and its contents are then placed in a test-tube which is exhausted through a side-arm until bubbles are seen to escape from the capillary, and when drying is complete air is admitted; the liquid at once rises into the capillary, followed finally by an air-bubble which seals it. The capillary is then sealed by heat and weighed in the usual way.

Liquids with High Vapour Pressures (*e.g.*, ether) are weighed [2] in a capillary (diameter, 1 mm.) in which is inserted a very fine hair-capillary, 20–30 mm. long, at a distance of about 30 mm. from the handle. The capillary is prepared and weighed as described and is dipped into a small deep dish containing the substance to be analysed, so that about 6 mm. of the wider portion of the capillary project. It is filled by raising it with heated forceps for a few seconds and then re-immersing in the liquid; on cooling, the liquid (3–4 c.mm.) enters the capillary, the fineness of which prevents evaporation. After 30 mins. on the balance no loss on weight should be observed; all warming must be avoided until the tube is sealed.

Very Hygroscopic Substances. In this case it is necessary to weigh both the empty and the filled boat in a weighing-bottle (Fig. 11), the handles of which are as thin as possible so as to minimise the effects of handling. The weighing-bottle should always be kept in the balance-

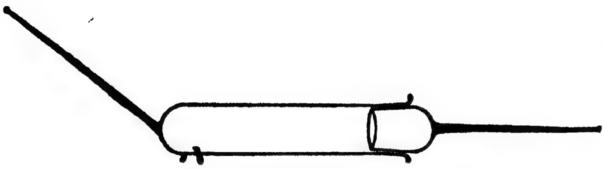

FIG. 11. Weighing bottle for hygroscopic substances. Actual size.

case when not in use; it should neither be placed in a desiccator nor heated, because the amount of moisture deposited on its relatively large surface must always be kept the same; it must also be constant in weight before weighing.

With extremely hygroscopic substances, however, absorption of water may still occur even under the above conditions, and a high-vacuum micro-desiccator (see below) must be used.

DRYING METHODS

This is a section of special importance, inasmuch as drying on the micro-scale is considerably easier to control (as well as being more rapid) than macro-scale operations; the use of vacuum methods and a current of dry air can contribute further to these advantages. The particular method adopted depends on the nature of the solvent to be removed, and on the melting-point or decomposition-temperature of the substance; those which decompose readily must be dried at the lowest possible temperature, and the adhering solvent is best removed in a high vacuum.

For drying at ordinary temperatures the boat containing the weighed substance is best placed in a desiccator which contains the appropriate drying agent, and is evacuated either by a water-pump or by high vacuum. The substance is constant in weight when, after drying for at least an hour, its weight is unaltered to within $\pm$ 0·002 mgm.

Pregl's Micro-Desiccator (Fig. 12) consists of a glass tube (length, 240 mm.; external diameter, 10 mm.), which is narrowed to a hair-capillary in the centre. One half contains several layers of firmly compressed cotton-wool, followed by 50 mm. of

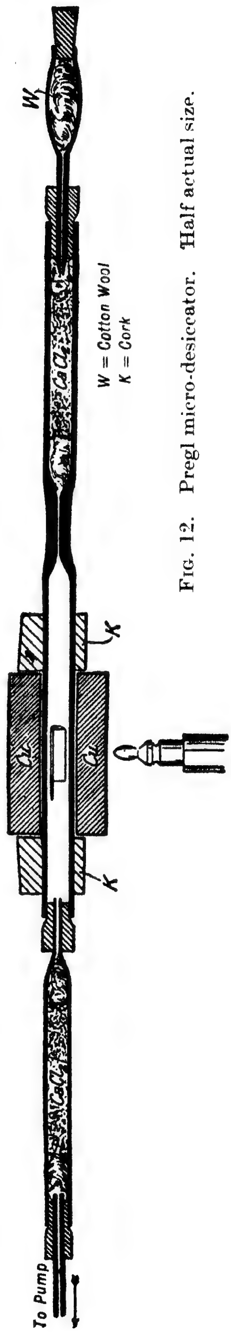

FIG. 12. Pregl micro-desiccator. Half actual size.

granulated calcium chloride which is held in place by a further layer of cotton-wool. The opening is closed with a rubber stopper, through which a thermometer capillary passes. This expands into an oval-shaped bulb, filled with compressed cotton-wool. The empty half of the main tube contains a boat (*e.g.*, as used for a combustion, p. 36). The other end contains a rubber stopper, through which is inserted the neck of a small tube filled with calcium chloride, which is connected to a pump. On evacuation the pressure in the micro-desiccator drops to the minimum attainable by the water-pump used, assuming that the capillaries are sufficiently fine ; they are intended only to permit a minimum (but constant) movement in the drying chamber by the entry of extremely small amounts of dried air.

The substance in the micro-desiccator is heated at any desired temperature by insertion of the latter in the copper block (regenerating block) used for drying halogen tubes (p. 91). In order to prevent the boat upsetting the tube is held tightly between two corks, which fit it exactly and so prevent rotation. Flat surfaces on both corks enable the micro-desiccator to lie on the bench without rolling.

After closing the rubber tubing leading to the pump with a screw-clip, the pump is turned off. After a few minutes, pressure equilibrium is completely restored, and the warm micro-desiccator can be transferred to the balance. The calcium chloride tube with its rubber tubing is then removed from the opening ; the platinum boat is withdrawn with a platinum hook, removed with the forceps, transferred to the copper block in the small desiccator, and weighed after 2 mins.

The drying "revolver" (p. 21) is also very useful. Since its temperature can be accurately fixed over relatively large intervals by the use of hot liquids of various boiling-points (alcohol, 78° ; water, 100° ; xylene, 139° ; dekalin, 188° C.) it is possible to dry 10 boats simultaneously in an aluminium groove 10–12 mm. wide, 7–8 mm. deep, and 250 mm. long. This also permits larger amounts of substances (to be used for a series of determinations) to be dried together.

The Pichler Drier (Fig. 13) is of special use for drying substances in beakers and Emich filter-sticks (p. 26). In this case the pump connection (V) is a long narrow tube which can slide through the rubber

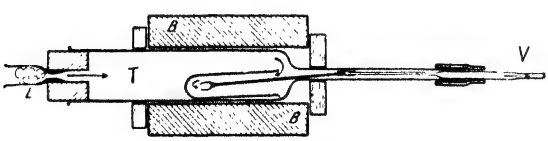

FIG. 13. Pichler desiccator.

tube, and out at the other end of the desiccator T. It is then attached to the end of a filter-stick as shown, and used to draw the stick (which is contained in its beaker) back into the main desiccator-tube (T). The wide portion of V makes the joint gas-tight. Heat may be used by enclosing T in a copper block, and dry filtered air may be drawn through L.

Fuhrmann [3] has devised heating blocks of different shapes for various purposes. Thus one (height, 1·8; diameter, 6–7 cm.) has a polished, platinised, slightly concave top surface, and is suitable for squat dishes. Another is a cylinder (depth, 2; internal diameter, 5 cm.) having a lid with holes for passing in an inert gas or hot air, or for applying a vacuum; all inside surfaces are platinised and polished. The third is a brass cylinder (diameter, 6 cm.), with an axial hole (depth, 40; diameter, 15 mm.) around which are eight holes (depth, 40; diameter, 10 mm.); this is used for evaporations prior to drying. Thermometer holes in the blocks provide for temperature control, and it is claimed that with a micro-burner this can be achieved to within 1–2° C.

High-Vacuum Desiccators. The Pregl type of micro-desiccator, however, does not suffice in all cases, because the dried substance is

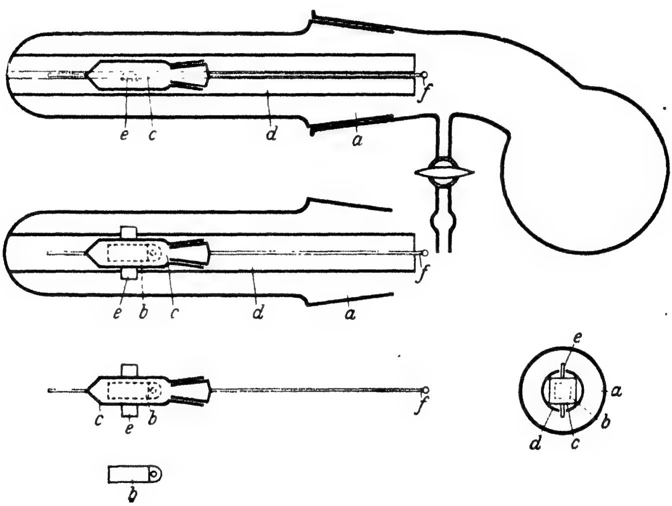

FIG. 14. Neumann high-vacuum micro-desiccator.

brought again into the moist air during transfer from the micro-desiccator to the weighing-bottle; and also because of the impossibility of maintaining a high vacuum for any length of time. Stoll and Wiedemann [4] describe an improved form, but owing to its instability this apparatus is difficult to manipulate.

In the Neumann high-vacuum micro-desiccator (Fig. 14) the substance is dried in a "revolver" desiccator (*a*) in a boat (*b*) in the weighing-bottle (*c*) under a high vacuum and at a raised temperature. The weighing-bottle is square in cross-section and slides in a device (*d*) inserted in the desiccator by means of two attached vanes (*e*), so that (*d*) and (*b*) remain horizontal and (*b*) cannot come into contact with the edges of the desiccator on withdrawal. The stopper of the weighing-bottle has a shaft (*f*) which projects beyond (*d*) and enables the weighing-bottle to be closed while it is in the desiccator.

The closed weighing-bottle and the boat are always weighed

together, but the final observation (especially after withdrawal from the desiccator) is not made until the object to be weighed is in a state of complete equilibrium (after about 15 mins.); after each weighing the zero of the balance is determined so as to eliminate errors resulting from possible displacements during the drying period. The long shaft of the stopper of the weighing-bottle is used to push it into the desiccator so that the vanes fit in the cavities of the sliding device, and then to open the bottle; the stopper is left inside (*d*). The closing cap of the desiccator is supplied with fresh phosphorus pentoxide and replaced, the vacuum is applied and the part of the desiccator containing the substance is heated in a drying oven as required.

The time of drying depends naturally, on the nature of the substance; in extreme cases several days and even weeks are required, particularly at low temperatures. After drying and cooling air is allowed to flow in through a tube filled with phosphorus pentoxide on pumice, and provided with a fine capillary at the entry end. The desiccator is then opened, and the weighing-bottle is closed, taken out of the desiccator and weighed; handling is done either with chamois leather or with freshly washed dry fingers.

METHODS OF ISOLATION AND PURIFICATION

Distillation as a method of purification of substances (*e.g.*, prior to analysis) usually involves fractionation. Where, however, simple distillation at normal pressures suffices, a 5-ml. distillation-flask of the usual design may be used with a test-tube supported under a tap as receiver; this may readily be adapted to steam-distillation.[5] The alternative apparatus shown in Fig. 15 is easy both to make and use; the overall height is 2·5–3·0 cm., and the volume of liquid used is 0·1–0·3 ml. The distillation arm contains a 3-mm. length of capillary tubing to prevent bumping, and the flask is heated over a micro-burner or by immersion in a water, glycerin or oil-bath; glycerin has the advantages for temperatures over 98° C. that its fumes are not unduly objectionable, and that it can be washed off the flask afterwards. The receiver is the bend in the side-arm, from which the distillate may be removed in a capillary-tube. With very volatile liquids it may be necessary to interpose a shield between the burner or bath and the side-arm, or to allow cold water to drip from a funnel on to the bend of the latter.

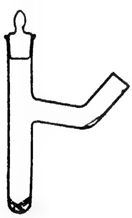

FIG. 15. Micro-distillation apparatus.

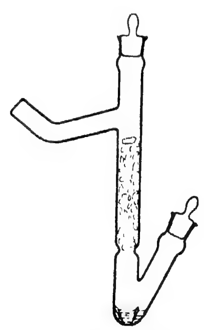

FIG. 16. Fractional micro-distillation apparatus.

Fig. 16 is a variation of this apparatus used for fractional distillation (overall height, 4·5 cm.)[5]; the fractionating "column" is filled with tiny pieces of glass; alternatively, the delivery end of the apparatus may be drawn out into several U-tubes, connected in series, which are

immersed in beakers at suitable temperatures for the fractional condensation of the different constituents of the substances present.[6] The receiver may, of course, be connected to a pump if it is desired to operate under reduced pressure. Pichler and Rachele [7] use a cold-point condensation method for quantitative fractional distillation (*e.g.*, of selenium bromide), but the method is too elaborate for many purposes. Another somewhat elaborate apparatus which, however, is noteworthy because it makes provision for the distillation of 0·5–2·0 ml. of sample into nine 0·1-ml. receivers under reduced pressure and without interruption, is described by Shrader and Ritzer.[8]

Capillary Tube Fractionation has been brought to a state of high efficiency by the work of Morton and Mahoney,[9] who have been able to obtain 106 fractions from a drop of liquid weighing 23 mgm. The fractionating vessel is conveniently a straight capillary tube (height, 15 cm. ; diameter, mm.) into which is packed finely ground glass wool. A constriction is made at about 3 cm. from the open end. The capillary is weighed, and a drop of liquid is placed in the open end and forced into the bottom of the tube by centrifuging the latter ; the tube is then reweighed to obtain the weight of sample. Wet filter paper is wrapped round the open end (Fig. 17), and the capillary is placed in an asbestos jacket and inserted in a copper heating block up to the constriction. The block is heated (the filter-paper being cooled with a jet of air) until 1 drop of liquid just appears above the constriction, and this may be removed by touching it with the open tip of a micro-boiling point tube ; the degree of heating should be controlled so that it is no more than is sufficient to provide a sample for collection, and for this reason it may be necessary to return the drop to the distillation capillary by centrifuging, and to re-heat it at a slightly lower temperature. With a little practice some 30 fractions can be collected from 1 drop, and their boiling-points determined separately (p. 187). Erdös and László's useful illustrated summary [10] of micro-distillation methods suitable for 0·1-ml. samples features, in particular, four types of micro-steam distillation apparatus.

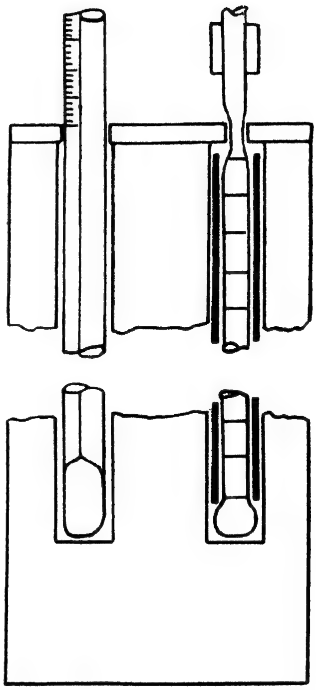

FIG. 17. Capillary tube fractionation apparatus.

Sublimation is a method of purification which lends itself admirably to micro-working when the substance in question is, of course, suitable ; it has been developed considerably in connexion with qualitative micro-analysis. The methods used are very simple. Thus, Eder's

apparatus shown in Fig. 18 is very effective, the sample being placed in the small projection in the base of a micro-beaker (height 2·5, diameter 1·0 cm.) and covered by a microscope cover-slip, on which the sublimate is deposited; a bath is used for heating, and provision can be made for carrying out the operation under a reduced pressure.

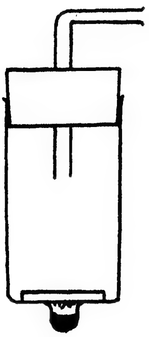

Fig. 18. Micro-sublimation apparatus.

It may also be desirable in some cases to cool the receiving surface, and this is easily arranged by directing the vapours on to the rounded end of a wide glass tube, through which cold water is conducted by means of an inner tube. The apparatus of Fig. 18 may be used in this way with a beaker about 1 cm. high and covered with a small glass dish containing ice-water, which is renewed as found necessary. The writer has also obtained good results in the separation of small amounts of volatile material from a relatively large bulk of sample, by the use of a horizontal melting-point tube, the closed end of which (containing the sample) is placed in a heating block, while the other is wrapped in damp filter-paper, and is kept cold by additions of drops of water; the sublimate may finally be isolated by breaking the tube at a point between it and the residue. In this method it is obviously important to avoid contamination during filling of the part of the tube on which the sublimate will form. This is ensured by filling the tube near one end and then sealing this end; or, if this is impracticable, by using a larger tube and inserting the sample through another thin glass tube which can just slide inside it. If it is necessary to work in a vacuum, the whole tube and contents may be placed in a larger tube, to which the vacuum is applied.

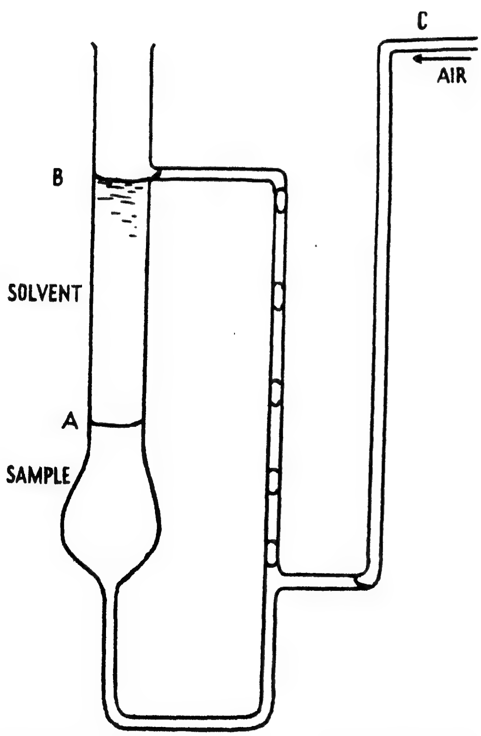

Fig. 19. Micro-extraction apparatus for liquids.

The same principles are the basis of the more elaborate apparatus described in the literature (*e.g.*, that of Clarke and Hermance [11]); refinements such as thermostatically controlled electrical heating methods are introduced.

Extraction and Crystallisation are conveniently considered together, because the former usually precedes the latter immediately. An efficient and yet simple apparatus for the extraction of liquids which tend to emulsify readily is that of Holt and Callow [12] (Fig. 19). *A* represents the level of the sample (*e.g.*, 5 ml.) and *B* that of the solvent, and bubbles of air (or inert gas) are produced in the arm by blowing into *C* at two to three bubbles per second. In this way portions of the sample are trapped between air-bubbles and blown over into the solvent, through which they fall on their way back to *A*.

For solids, one of the many types of micro-Soxhlet apparatus described in the literature may be used. Batt and Alber [13] have made a comparative study of a number of the more recent of them ; they point out that when the residue as well as the extract is to be determined, a percolation method in which the condensed solvent falls continuously through the sample (which is contained, for instance, in a sintered glass crucible) is preferable to the Soxhlet method, with its risk of loss of particles of sample due to syphoning. A simple apparatus for this type of work is shown in Fig. 20, which is self-explanatory. The outer tube is conveniently a 6-in. test-tube, and a condenser of the type shown in Fig. 83 (p. 197) may be used. For other purposes the conventional Soxhlet apparatus, suitably reduced in scale, cannot be bettered.

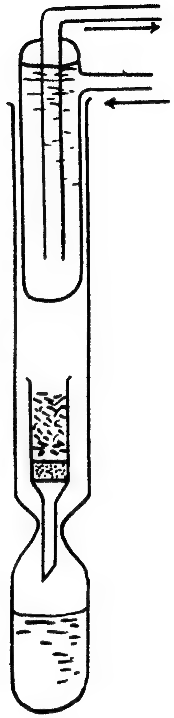

FIG. 20. Micro-extraction apparatus for solids.

Crystallisation calls for some special comments ; thus, although the methods are essentially those of macro-chemistry but on a reduced and simplified scale, difficulties arise owing to the high surface tension of small volumes of liquids (especially of drops), and the poor crystallisation which results owing to the selective rapid evaporation and creeping which occurs at the edges of the solution in a dish. Consequently, despite the simplicity of the apparatus and technique, considerable practice is necessary in order to obtain the best results.

Watch glasses or microscope slides with depressions may be used as crystallising vessels, and in some cases crystallisation from aqueous solutions is aided by the introduction of the vapour of a liquid in which crystals are insoluble (*e.g.*, alcohol, acetone, or ether) ; a convenient means of doing this is to place the solution to be crystallised and the volatile liquid between the same pair of Petri dishes in a warm place. Refinements have, however, been suggested for use especially where it is necessary to avoid mechanical losses. Blount's apparatus for combined extraction and recrystallisation (Fig. 21) is an example [14] ; it comprises a sintered glass filter crucible (which contains the sample)

suspended from the end of a vertical condenser on a platinum wire. The end of the condenser has a ground glass joint which enables it to fit into the neck of a flask (diameter, 3 cm.) which contains the solvent. Extraction takes place on heating under reflux, after which some of the solvent is evaporated if necessary, and the flask is allowed to cool so that crystallisation can take place. If desired the crystals may be returned to the crucible for re-extraction. It is also often convenient to arrange that the crystals are formed in a vessel suitable for their direct separation from the mother liquor subsequently, *e.g.*, in a centrifuge tube, or on a filter (see below).

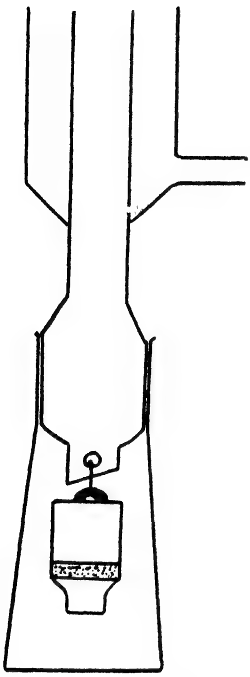

FIG. 21. Apparatus for micro-extraction and recrystallisation.

Separation of Solids from Liquids is usually achieved by filtration, although there are circumstances when the centrifuge is better ; a suitable centrifuge for quantitative gravimetric work on the micro-scale is described by Langer.[15] Quantitative filtration (for gravimetric analysis) is dealt with on pp. 88 and 102, and some qualitative methods are similar in principle. In particular, apparatus containing a sintered-glass filter medium of appropriate pore-size is finding increasing use on account of its convenience. One drawback of this type is the difficulty of recovering the separated solids from the filter, especially if this is inserted in a narrow tube.

FIG. 22. Simple micro-filter.

An alternative which is suitable for many types of quantitative work is shown in Fig. 22, and is easily made ; it consists of two pieces of glass tubing with a piece of hard filter paper sandwiched between them, and held closely together by stretched rubber tubing. The upper tube (diameter about 1 mm.) is drawn out to a truncated cone, the external diameter of which is exactly the same as that of the lower (*i.e.*, capillary) tube. Such a filter will fit into the stopper of an ordinary filter flask ; it will stand up to quite a high vacuum, and may be dismantled for cleaning or for recovery of the precipitate. If the precipitate is to be ignited or titrated, the paper and its contents are transferred directly to the crucible or titration vessel, any residue on the glass being wiped off with a scrap of filter paper which is also added.

The Emich filter-stick (Fig. 23) is also very convenient, especially when it is difficult to avoid losses on transference of the precipitate from the reaction-vessel to the filter. It consists of a glass tube about 4 cm. long, with a wide end into which is sealed a filtering surface such

as a sintered porcelain or glass or spongy platinum plate. When used for quantitative work (as described by Briscoe and Matthews [5]) the small beaker containing the sample is first weighed with the filter (which is placed on the balance pan) outside the beaker. The sample is then dissolved and precipitated as required, the filter stick is inserted, and the liquid portion is removed from the beaker by suction as shown. The wash liquors used subsequently are dealt with similarly, so that eventually the filter-stick, precipitate and beaker may be dried and weighed "en bloc." The method can, of course, be used only in this way if the final wash is with pure water; quantities of up to about 10 mgm. of precipitate in 10 ml. of liquid can be dealt with.

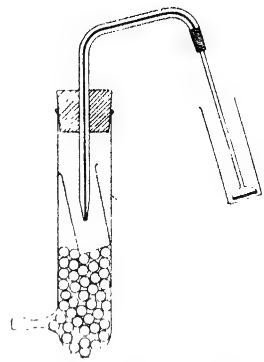

Fig. 23. Emich filter-stick.

Chromatographic Adsorption.[16] A reference should be made to this new and useful method of separating mixtures of organic compounds, since it can be conveniently operated on the micro-scale. It is based on the discovery of Tswett (1906), who poured solutions containing plant pigments through a vertical tube packed with an adsorbent and found that certain constituents of the solution were adsorbed selectively, forming horizontal bands down the column. As a later development it became possible to recover each constituent separately by pouring an appropriate solvent through the column (elution).

The method has been developed along novel lines and applied to two liquid phases by Martin and Synge.[17] Their method is best illustrated by an example, namely, the separation of amino acids. The adsorbent is silica gel, which is first impregnated with a solution of methyl orange, dried, suspended in chloroform and poured into the column. When all the solvent has drained out, a solution of the sample in chloroform and butyl alcohol is poured in, and the chromatogram is developed by addition of fresh solvent. The positions of the acids are shown by the indicator, and their separation may be achieved as completely as possible by adjusting the alcohol content of the solvent, since the amount of any one acid in a given length of column depends on its partition coefficient between the two

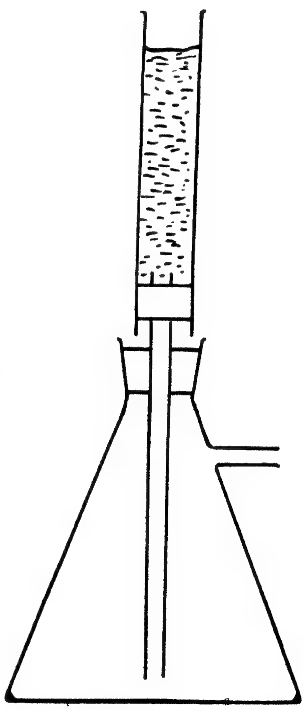

Fig. 24. Micro-apparatus for chromatographic adsorption.

solvents used. In this way it has been possible to recover amino acids from mixtures to the extent of 80–100% of the amounts actually present. The method is obviously capable of wider applications. The ordinary methods of adsorption may also be aided by electrophoresis produced by the insertion of electrodes at the top and bottom of the column.

The technique and many applications of the method are well described by Zechmeister and Cholnoky.[16] Columns suitable for micro-work are about 3 cm. long and 1–3 mm. in diameter, and will adsorb down to about 0·1 mgm. of material (see Fig. 24).

Unfortunately, it is not possible to make general recommendations which will cover all (or even most) of the examples likely to be encountered; each must be treated as a separate problem, and the best adsorbent, solvents and operating conditions ascertained. A few guiding principles may, however, be indicated. Thus, although the many hundreds of compounds tested have revealed no "universal" adsorbent, the oxides, hydroxides and salts of the alkaline earths are best for the purpose, especially if the particle size does not exceed 200-mesh; small-scale tests with various adsorbents will usually settle this point. The selections of solvent, adsorbent and eluant are to a great degree interdependent. Adsorption is greatest from saturated hydrocarbons, but they should be pure and dry. Most liquids more polar than the adsorbed substance will elute it; acids and bases are very effective.

The separated constituents are located in the column by inspection in visible or in ultra-violet light, by brushing an appropriate agent on to the column, or by using an adsorbent which gives coloured reaction-products with one or more of the constituents.

The method proves of greatest value in microchemical analysis for the quantitative separation of mixtures,[18] as an aid to purification, and for concentrating the constituents of very dilute solutions. If analytical methods are applied to the eluate, it is a wise plan to check the method against standard solutions of the substance being determined after treatment by the same adsorption and elution procedures. Some recent examples of interest illustrating its application are the separation of sugars as their azoyl derivatives,[22] and the resolution of enantiomorphs.[23]

VOLUMETRIC METHODS

Although such methods probably find their principal applications in inorganic microchemical analysis, there are a number of examples in this book for which micro-titration is necessary. Moreover, where quantities too small to be weighed accurately on a microchemical balance are to be measured out, this may be often done volumetrically by taking an aliquot portion of a suitably-diluted standard solution of the substance. The use of dilute standard solutions provides one method of titrating small quantities, but it is usually limited by the

indefinite end-points and by the solubility effects obtained under such conditions. Micro-titrations are, therefore, usually carried out with small volumes (*e.g.*, 5–10 ml.) of solutions of normal strengths (*e.g.*, 0·1–0·01 *N*).

Micro-Burettes may be 1–10 ml. in capacity, with 0·05-ml. sub-divisions, and they often have Schellbach scales (Fig. 25). Jena or similar glass is preferable to ordinary glass, in that it yields no alkali to the contents even after years of use. Readings are made with an adjustable lens, and the burettes are supported in a titration stand with a horizontal and a vertical pane of opal glass in front, to facilitate the observation of the end-point. The standard solution is pumped into the burette from the 500-ml. storage vessel, through a glass tube which ends in a capillary exactly at the zero of the burette; immediately the pressure is released, therefore, the solution standing above the mark automatically syphons back again into the storage vessel.

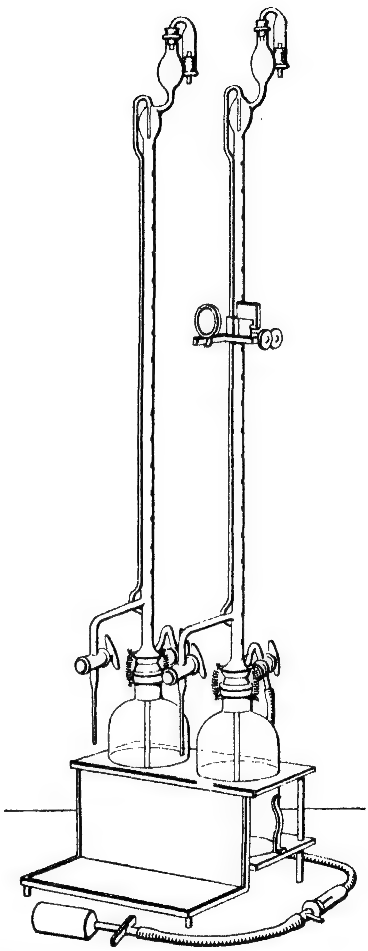

Fig. 25. Micro-burettes with devices for filling and automatic zero standardisation.

The solutions are protected from atmospheric carbon dioxide by means of small tubes of soda-lime, which must frequently be renewed. Pregl recommends that rubber tubing should be used to connect the burette with its jet outlet, and that the latter should be operated by means of a pinch-cock or by pressure on a small glass ball in the rubber tubing. Glass cocks fused obliquely on to the burette are preferred by some workers. Both methods allow 0·01 ml. to be withdrawn without difficulty, after some practice, but the former is certainly the better for use with alkaline solutions. In order that, on the one hand, standard solutions may be run out rapidly, and on the other, that very small drops may be withdrawn with a high degree of accuracy, the capillary tap outlet is 6–8 cm. long and 0·3 cm. in diameter, and is drawn out to about 1 mm. diameter at the lowest part only. Thick-walled capillaries drawn out are preferable on account of the smaller risk of breakage and the easier manipulation; they are ground to a blunt cone at the outlet.

The limiting factor of the accuracy under ordinary conditions is, of course, the smallest volume of liquid which can be delivered at one time from the jet, and this is determined by the size of the drop which, in turn, depends to a great extent on the surface tension of the solution used. This difficulty is usually overcome by working with the tip of the jet in contact with the side of the titrating vessel during the titration, so that drops do not form. It is preferable (if other conditions allow) to have the tip actually immersed in the solution being titrated. A rapid stream of small bubbles of an inert gas (*e.g.*, nitrogen) is very suitable for stirring. Other points of technique are dealt with by Thornton [26] and by Linderström-Lang and Holter,[19] and Wyatt [24] has recently described a micrometer-controlled burette delivering 0·0004 ml.

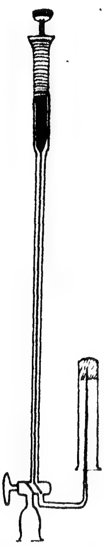

FIG. 26. Piston-type micro-burette.

Other and simpler types of micro-burette exist. Thus, a suitably sub-divided 5-ml. pipette may be converted into a micro-burette by the addition of a cock having a suitable jet. Difficulty is, however, sometimes experienced in filling micro-burettes which are not specially equipped for this purpose (as are those shown in Fig. 25), owing to their small diameters ; where the nature of the solution permits it may, with a little inconvenience, be drawn into the burette *via* the jet by suction applied at the top.

Where it is necessary to add at one time smaller quantities of liquid than can be controlled by either a tap or a ball in rubber, any form of tap must be dispensed with completely. The best substitute (Rehberg) works on the piston principle, and is shown in its simplest form in Fig. 26. The burette contains a column of mercury, the level of which may be raised or lowered by the screw at the base. In this way the titrating solution may be drawn into the burette (through the small funnel, *via* the right-hand base of the glass tap), and forced out again (through the other bore of the tap) into the solution being titrated. It is apparent that very fine jets may thus be used ; that by turning the screw through only a very small angle, a very small volume of liquid is expelled ; and also that the screw may be calibrated so that the angle through which it is turned is a measure of the volume expelled. Refined forms of this burette have an air-interface separating the mercury and the solution.

Micro-Pipettes are conveniently made by drawing out lengths of glass tubing to the desired dimensions and calibrating them ; 1·0–0·1 ml. are convenient capacities. They present no special features of design, being smaller versions of ordinary macro-pipettes, but a few points of importance arise in their use. Thus, they should be used only with solutions having a similar viscosity to that of the liquid used to calibrate them (*i.e.*, usually water) ; if necessary they must be specially calibrated with the solution for which they are actually to be used.

Under such circumstances it may in fact be more convenient to use a micro-burette as a pipette, or a pipette (Fig. 27) which contains (as distinct from delivering) the desired volume, the residues of the contents being washed out of the pipette with water after drainage is complete ("auswaschen method"). This point arises by reason of the very slow drainage rate of micro-pipettes, which is due to their very fine jets.

Fig. 27. Micro-pipette containing a definite volume.

The Pregl micro-pipette (Fig. 28) is calibrated by weighing with mercury ; in the test certificate supplied, is stated the accurate volume, the weight empty, and also the volume which runs out up to within 1 mm. of the capillary ; it is not absolutely necessary to fill exactly to the mark. The diameters of the capillary and the tip are so chosen that by reason of the surface tension and viscosity, the filled pipette may be held vertically without the solution running out. The solution may be measured to within $\pm$ 0·0001 ml., but because the pipette is calibrated for delivery, the residue on the walls must be rinsed out with water from the wide end.

Fig. 28. Pregl micro-pipette. ½ actual size.

A precision weighing-pipette is illustrated on p. 211 (Fig. 89).

Micro-Diffusion is a volumetric method of limited application, but it is worthy of mention because it has been used successfully in many organic micro-analyses (*e.g.*, for the determination of Kjeldahl nitrogen after digestion, for volatile amines, and for urea in blood, etc. [20, 21]). The principle of the method is the formation of a volatile reaction-product in the outer circular chamber of the apparatus shown in Fig. 29 ; and its subsequent diffusion into and reaction with the reagent in the inner circular chamber. The whole apparatus is sealed by a flat glass plate and a suitable fixative (*e.g.*, vaselin).

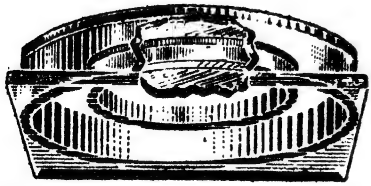

Fig. 29. Micro-diffusion apparatus.

Since the walls of the inner vessel are less than half the height of those of the outer vessel, diffusion consequently occurs relatively rapidly, especially if it is aided (*e.g.*, by heat, agitation or reduced pressure). As an example, for the determination of urease in blood 0·2 ml. of sample in the outer chamber is fermented by urease, and the resulting ammonia is subsequently liberated by addition of 1 ml. of saturated potassium carbonate solution. Excess of standard acid is placed

in the inner vessel, the lid is put on, and in due course the acid is back-titrated.

Wyatt [25] gives a useful survey of recent micro-volumetric apparatus, with 67 references ; he deplores the fact that most of the apparatus described is not commercially available in this country.

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CHAPTER III

DETERMINATIONS OF THE ELEMENTS

DETERMINATION OF CARBON AND HYDROGEN

PREGL'S METHOD

THIS method, first studied by Pregl in 1910, may be regarded as the classical procedure of microchemical analysis. However, since its publication in 1912, it has been the subject of numerous further investigations by Pregl's pupils and others.[1-3] These have had the object of simplifying the technique, and of minimising the errors necessarily inherent in a determination of this kind. Such errors are mainly of three types, namely, those inherent in the apparatus, in the purity of the reagents, and in the manipulation. In addition to modifications of Pregl's method other micro-methods have been suggested, and these are preferred by some workers and are dealt with further on p. 60. On the whole, however, the basic principles of Pregl's method still stand firmly, and the method is more widely used than any other. It will therefore be described in detail, together with those of the modifications which have proved definitely advantageous. Roth states in the last edition of this book that he has been able to work to within $\pm$ 0·2% in determinations made on about 5,000 different substances. Many workers would place the average error nearer to $\pm$ 0·3% for carbon or for hydrogen, and if the following directions are followed closely there should be no difficulty, after a little practice, in attaining at least this degree of accuracy. It is, however, a good plan for the novice to acquire the technique by first working on the " semi-micro " scale, *i.e.*, with 10- to 15-mgm. samples.

The Principle of the Method is essentially the same as that of the corresponding macro-procedure, *i.e.*, the classical Liebig method in which the sample is heated in a stream of oxygen in presence of an oxidising catalyst, and the resulting carbon dioxide and water (produced from the carbon and hydrogen, respectively), are absorbed (*e.g.*, in soda-lime and calcium chloride, respectively) under conditions which enable them to be determined, usually by weighing. The principal departures from the classical method are concerned with the catalyst used in the combustion tube, which may now be regarded as universal in that it reacts satisfactorily with almost any substance; in the careful adjustment of the speed and pressure of the oxygen through the system (p. 52); and in the necessity for close attention to technique and certain details, such as the use of matured or treated rubber tubing for connexions.

Apparatus

The Committee appointed by the American Chemical Society to deal with the standardisation of microchemical apparatus has published

specifications for this apparatus [4]; they follow Pregl's method closely. The complete Pregl apparatus is shown in Fig. 30, and the individual items will now be dealt with separately.

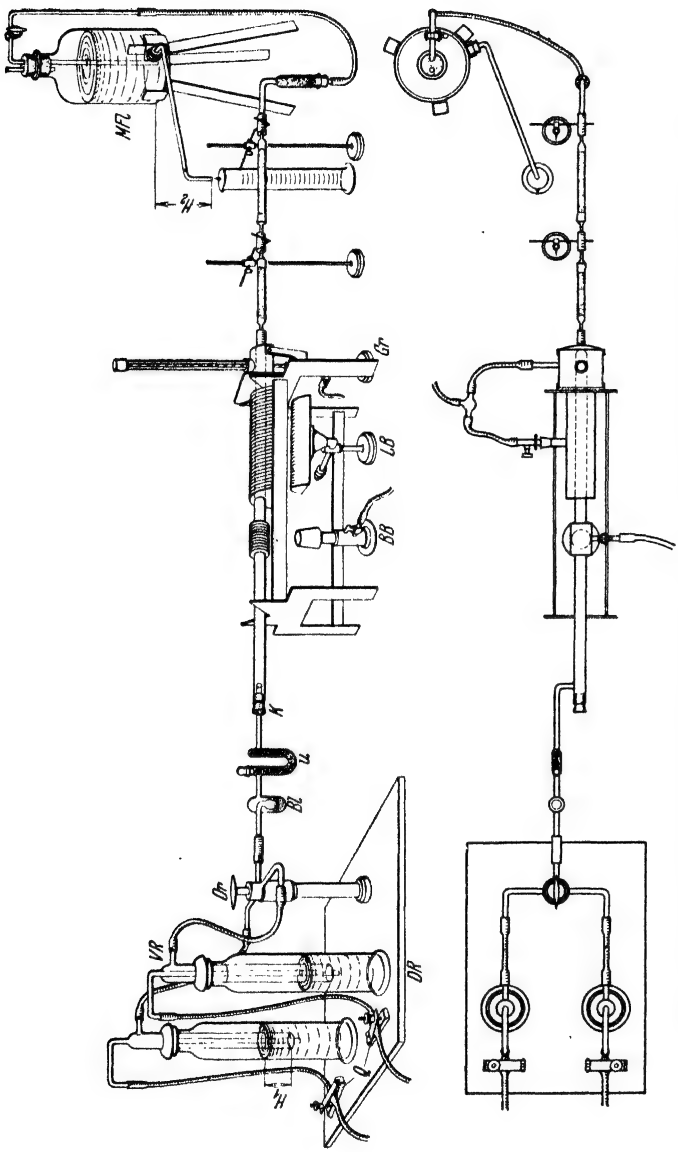

FIG. 30. Elevation and plan of apparatus for micro-determination of carbon and hydrogen. One-twelfth actual size.

Pressure Regulator (*DR*). This is mounted on a board, and comprises essentially two bell-type gasholders, one of which is used for introducing the air and the other for oxygen. Each is a glass bottle (height, 240; diameter, 60 mm.) about two-thirds filled with 5% sodium hydroxide solution, and closed with a wooden cap through which passes the bell of the gasholder; it is held in position by three

metallic springs. The bell is a wide glass tube (length 200, diameter 20 mm.) in which a narrow glass tube (diameter, 3–4 mm.) is sealed so that its lower end reaches 6–7 mm. beyond the open end of the wide tube. The inner tube is the inlet for the gas, and is connected to the appropriate gas holder through a matured rubber tube provided with a precision pinchcock (Q in Fig. 30, and Fig. 31). The gas passes out through a glass tube sealed in the side of the upper end of the bell-tube, to the corresponding limb of the three-way cock Dr, which enables oxygen to be replaced by air after the complete combustion of the material; or the supply of gas to be cut off.

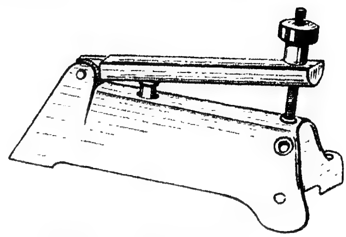

FIG. 31. Precision pinchcock. Half actual size.

This arrangement ensures that the air or oxygen is delivered continuously in small bubbles. The pressure of the escaping gases is determined by the difference in levels (H_1, Fig. 30) between the liquid in the inner and outer vessels, and cannot exceed that corresponding with the maximum value of H_1. The velocity of the gas in the combustion tube is also dependent solely on H_1 and, therefore, can be adjusted by raising or lowering the moveable bell-tubes. It is obvious that, for reasons of economy, the flow of gas into the pressure regulator should be reduced (by means of the needle-valve and/or the precision pinchcock) until the necessary pressure-difference is just maintained and the gas-bubbles are evolved at intervals of at least 2–3 secs. If the apparatus is used in a room which cools down considerably overnight (*e.g.*, in heated rooms in winter) the sealing liquid of the bell gasholder may be sucked back into the gas inlet tube. In such cases the central inlet tubes of the gasholders are provided with a sufficiently large bulb.

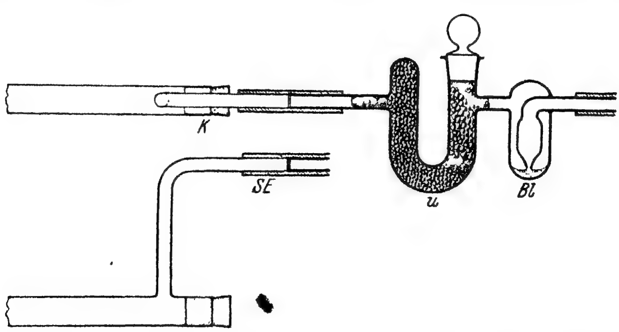

FIG. 32. Method of connecting U-tube (u) to side-tube (SE) of the combustion tube; Bl is the bubble-counter.

U-Tube with Bubble-Counter. The gases pass from the 3-way cock through a tube (length, 35–40; external diameter, 5 mm.) into a bubble-counter (Bl, Figs. 30 and 32). As shown, the inlet tube widens into a bulb and ends in a fine tip (diameter, not exceeding 1 mm.) which

is 3–5 mm. above the bottom. The escaping gases pass into the U-tube (length, 120–140; external diameter, 12 mm.) fused on to the side of the bubble-counter, and having a ground-in stopper in the arm attached to the bubble-counter; the other arm is closed. The outlet tube is connected with the side-arm (*SE*) of the combustion tube through 50 mm. of rubber tubing, glass being in contact with glass.

The U-tube is cleaned successively with chromic acid, water and alcohol, and dried in an oven. A small wad of cotton-wool is inserted in the side-tube of the sealed arm, and granulated anhydrous calcium chloride is added until only about one-third of the right tube is empty. A small wad of cotton-wool holds this filling in position, and the empty portion of this arm leading to the bubble-counter is then filled with air-dry soda-lime up to the level of its side-tube. A wad of cotton-wool prevents the soda-lime from falling into the bubble-counter (see Fig. 32). The glass stopper is carefully warmed and held in its ground joint with Krönig cement (p. 45). A fine pipette is used to introduce into *Bl* sufficient sodium hydroxide solution to cover the tip of the delivery tube by about 2 mm.; any excess is removed by blowing into the other end. The apparatus is cleaned with cotton-wool, and suspended on a wire stirrup from a stand.

Combustion Tube and Furnace. The combustion tube is of hard glass; length (exclusive of the neck), 500; external diameter, 9·5–10·5 mm. The special types of glass made for the purpose will last for about 250 analyses; quartz is unnecessary, and it soon becomes opaque. The gases enter through a right-angled side-tube [5] at about 18 mm. from the mouth (Fig. 30), so that the bubble-counter need not be detached every time the combustion tube is filled.

At the other end of the combustion tube is a neck (length, 23–25; external diameter, 3·3–3·5; aperture, 2·0–2·5 mm.). It is advisable to form this neck, not by drawing out the tube, but by carefully sealing on a tube of the necessary dimensions. The end of the neck is ground even and perpendicular to its axis with fine carborundum powder; the edges are rounded in a flame, without restricting the bore. The tube is protected from direct contact with the flame of the tube burner (*LB*, Fig. 33), and from distortion, by surrounding it with a roll of fine-mesh wire gauze (*El*) 180 mm. long. A similar roll (*Ek*), 50 mm. long, surrounds the tube over the moveable burner. The entire filling of the tube is maintained at red-heat by the regulable tube burner (*LB*) in a combustion tube stand 250 mm. long, and of such a height that the tube resting in the V-shaped notches is at the same height as the delivery tube of the three-way cock. Along the edges of both sides are narrow parallel gutters (*R*), which carry a coarse piece of iron gauze (T), bent to a U-shaped section; this forms a tunnel-shaped space 180 mm. long, and encloses the filled portion of the tube.

Electricity is preferable to gas for heating the tube filling,[3,6] because the worker is exposed to less heat of radiation; the laboratory air is not affected by the gases evolved (steam, in particular, can

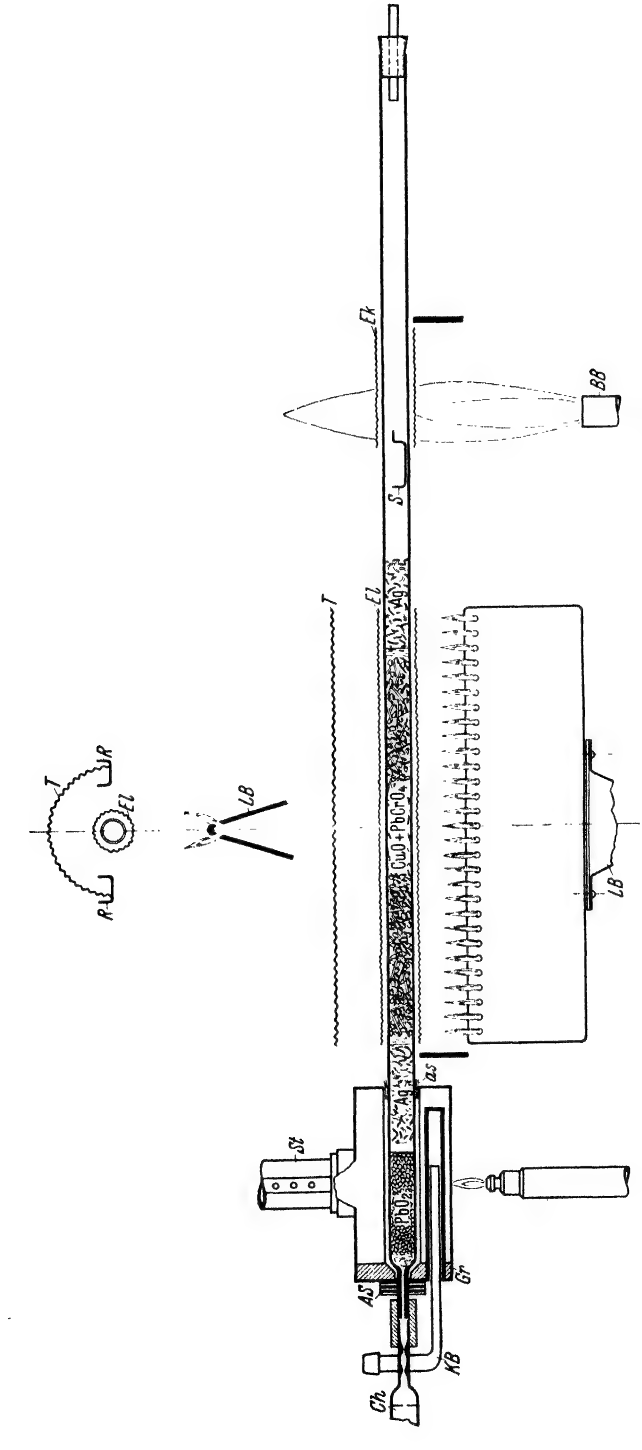

FIG. 33. Filled combustion tube in position. One-third actual size.

influence the carbon value); and the temperature of the tube may be controlled to within $\pm$ 10° C. The use of gas also almost invariably means that by the evening the temperature of the room is higher than that of the balance room. A moveable electric burner is excellent where substances having the same or very similar properties are continually being analysed. In many laboratories, on the other hand, combustion cannot be carried out to a fixed schedule, but may have to be varied according to the type of substance. It is, therefore, preferable to use the moveable gas burner, since the substance is then under constant observation, and the temperature can be regulated much more conveniently.

Heating Mortar. The lead peroxide used (see p. 44) has the property of retaining water, even at high temperatures. In order to obtain correct hydrogen values, therefore, it is necessary to maintain it at a constant temperature, not only during the combustion, but also when the tube is being pre-heated. This is done by means of a heating mortar (*Gr*, Fig. 33). The early mortars consisted of solid cylindrical copper mantles, heated at 180° C. by a micro-burner, a thermometer being let into the top. Because of their simplicity they are still to be recommended where a constant gas pressure is available.

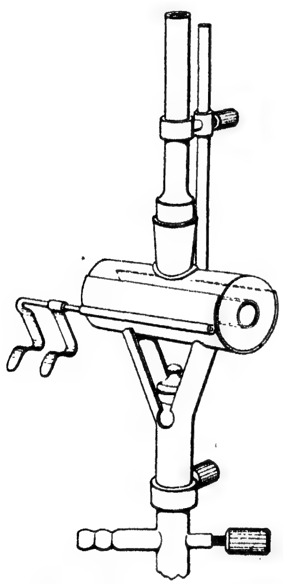

FIG. 34. Gas-heated mortar.

Where the gas pressure varies, hollow mortars containing a boiling liquid (*e.g.*, cymene, b.p. 176°, or dekalin, b.p. 188–190° C.) are now generally used. The Verdino [7] type used in conjunction with the gas-heated tube burner (Fig. 34) has proved satisfactory in almost daily use during four years; leakage is prevented by tightening a screw, and the resin deposited after prolonged use is easily removed by pyridine bases and benzene. A possible disadvantage is that the liquid cannot be seen boiling. Lieb [8] recommends a Jena-type glass heating mortar (Schöbel design) having the shape and dimensions of Pregl's hollow metal mortar. The hollow cylinder (length 70, external diameter 35 mm., capacity 40 ml.) surrounds a central chamber (diameter, 14 mm.; 6 mm. where the neck of the combustion tube passes through). In the top of the mortar is a standard ground-glass joint for an upright tube (length, 350 mm.). The hollow space is about half-filled with dekalin containing a few scraps of pumice. The mortar fits into a semi-cylindrical metal groove, lined with asbestos, and is clamped by the vertical tube to a glass rod fixed vertically in the groove; the copper heater is fixed in a metal tube soldered on to the groove, and a gas micro-burner is built into the support.

Electrically heated mortars are seldom favoured, because of the difficulty of attaining a constant temperature over long periods ; this arises from the fact that the heat supplied is constant and in excess of that lost by radiation, so that the temperature gradually rises. This difficulty, together with those arising from a tendency to overheating due to the gumming of the boiling liquid and to the inflammability of the boiling dekalin, is overcome (by Kirby [9]) by the use of mercury as heating liquid in a Schöbel mortar ; this, of course, will not gum, and it lends itself to temperature control to within $\pm$ 0·25° C. if a platinum wire contact is inserted in the vertical tube (Fig. 35). As the temperature rises, contact is made between the wire and the mercury, and the heating current is cut off ; it comes on again as soon as the mercury falls and contact is broken.

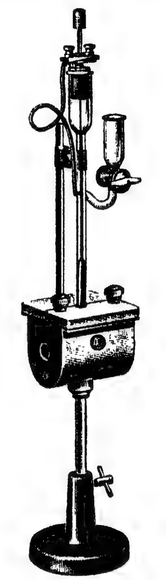

FIG. 35. Electrically-heated mortar.

Connexions. Glass joints are preferable throughout the apparatus ; they are usually essential if 2 mgm. or less of sample are taken.

The rubber tubing used to connect up the apparatus from the gasholder to the combustion tube was investigated exhaustively by Pregl and, later, by Böck and Beaucourt [10] and by Boetius.[11] Consequently, it is no longer a serious source of error. Errors were established by Pregl only for inadequately matured tubing, *e.g.*, new tubing which still evolves organic solvents or tubing through which ordinary coal gas has previously passed (*cf.* p. 58). The following " artificial ageing " (*cf.* Friedrich [2]) is recommended by Pregl : New indiarubber tubes (bore, 3·5–4·0 ; thickness, 1·0–1·2 mm.) are immersed in 40–50% potassium hydroxide solution at 60° C. for 2 hrs., and steam is passed through them for 2 hrs. It is advisable to steam out the tubes of the apparatus at least twice a year. For longer connexions well-joined glass or lead tubes are recommended.

The connexion of the calcium chloride tube to the neck of the combustion tube and to the soda-lime tube requires special consideration, because ordinary indiarubber is unsuitable. It is hygroscopic ; it is very often porous, or becomes so in use ; and it is permeable to carbon dioxide. Its hygroscopic properties first became evident from the blank tests carried out without any precautions, in which the increase in weight of the calcium chloride tube was found to be about 0·1 mgm., even when the potassium hydroxide apparatus showed no increase.

Experiments led finally to a method of impregnation of rubber which reduced these drawbacks to such an extent that even without regulation of the pressure by a Mariotte flask (p. 41) it is often possible to obtain accurate results. Thick-walled, smooth absorption tubing (external diameter, about 8 ; bore, 2·0–2·5 mm.) is used. A narrower

bore is unsuitable because the tubing is strained when it is being fitted over the glass tubes. Lengths of 20 and 25 mm. (for the combustion and soda-lime tubes, respectively) are placed in pure molten paraffin wax in a small flask, which is evacuated with a water pump on the boiling water-bath. As soon as the contents cease to foam, air is re-admitted to force the molten paraffin into the finest interstices of the rubber. Evacuation and re-admission of air are repeated until at the highest attainable vacuum no further bubbles are seen on the tubing ; this requires 30–60 mins. The tubing is drained while warm, wiped on the outside, and on the inside by means of cotton-wool wound round steel wire. When in use the tubes are often wiped inside with a small wad of cotton-wool wound round a wire and moistened with a trace of glycerin, and then with a wad of dry cotton-wool (free from loose fibres) to remove any excess of glycerin.

The freshly treated tubing is wrapped in a spiral band of tinfoil (width, 6–7 mm.), and then in well-sized paper, and stored in a desiccator over phosphorus pentoxide.

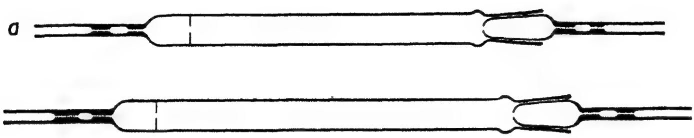

FIG. 36. Absorption tubes. (*a*) Calcium chloride tube. (*b*) Soda-lime tube. Half actual sizes.

The Absorption Apparatus. After the combustion tube this is the most important part of the whole apparatus, because the weights of water and carbon dioxide formed are so very small in relation to that of the absorption apparatus. The exact dimensions of the apparatus are of special importance, and even with apparatus from reputable firms, the bores of the capillaries should be checked with platinum or silver wire, the diameter of which is determined (with a micrometer screw-gauge) to within $\pm$ 0·02 mm.

The Calcium Chloride Tube (Fig. 36, *a*) is a thin-walled glass cylinder (length, 90 ; external diameter, 8–9 mm.), constricted at one end, and having at the other a ground-in stopper 12–14 mm. long. An empty space separated from the calcium chloride layer by a thin diaphragm having a central perforation (diameter, 0·3–0·5 mm.), is left in the entry end ; this and the capillaries produce a variable diffusion-potential, which ensures little variation in the weight of the apparatus. If the perforation is obstructed by water (*e.g.*, in the combustion of substances which have a very high hydrogen content), the water must be vaporised by warming the diaphragm, or the pressure of the system raised by moving back the burner about 30–50 mm. The connexion for the calcium chloride tube (external diameter, 3·3–3·5 ; length, 25–30 mm.) is constricted into two capillaries, (diameters, 0·1–02 mm.). At the exit end of the calcium chloride tube,

a ground-in hollow stopper 10–12 mm. long forms a second antechamber. This connects with the absorption tube.

The Soda-Lime Tube (Fig. 36, *b*) is exactly like the calcium chloride tube, except that the chamber is 100 mm. long, so that a large number of analyses may be made without re-filling.

The Principal Sources of Error inherent in absorption tubes are the increase in weight due to access of moist air, the presence of static electrical charges on the glass, and the decrease in weight which results from the displacement (by diffusion) of the oxygen by air. These effects may be accentuated if the balance room is at a different temperature to that of the room in which the combustion is being carried out. Diffusion effects have been minimised by the insertion of fine platinum wires in the capillaries, by providing a mercury or steel ball seal to close the capillaries,[12] or by the use of a hollow ground-glass joint containing a hole, which may be turned so as to open or close the tube. Many workers, however, prefer simply to weigh the tubes filled with oxygen and unsealed, since diffusion occurs very slowly and, if the weighing procedure is standardised, its effect is negligible [13]; similar tubes full of oxygen may be used as tares (see p. 13).

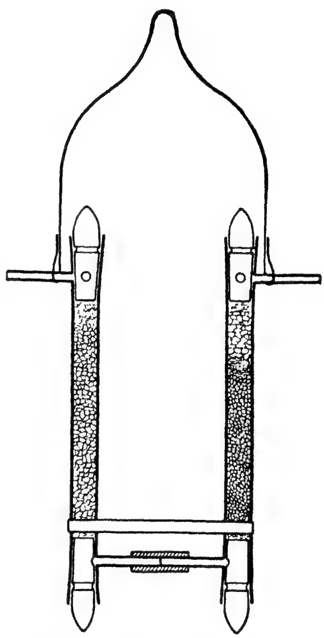

FIG. 37. Flaschenträger's absorption apparatus.

Flaschenträger's Apparatus (Fig. 37) should be mentioned because it embodies certain of the modifications outlined above, and it is widely used.[14] The method of operating the sealing stoppers is apparent from the illustration, and in order to prevent the lubricant from working out, the stopper has a ring-shaped groove (width, 2; depth, 0·5 mm.) 5 mm. from the bore. The lower part only of the stopcock is greased, with lanolin (adeps lanæ), or with a mixture of vaselin and beeswax (3 : 1). A disadvantage is that the tubes are not easily wiped, and the stopper may therefore come out during this process. If the tubes are opened after releasing the excess oxygen pressure, they lose about 0·1 mgm. During the analysis the absorption tubes are held together by a rubber band; they are hung up on a small stand by a wire stirrup, and are protected by asbestos shields from the heat radiated from the mortar. The Friedrich absorption apparatus is similar,[15] but simpler. Other designs in common use are referred to by Sternberg.[16]

Pressure Relations in the Apparatus, and the Use of the Mariotte Flask (*MFl*, Fig. 30, and Fig. 39). The gas pressure, corresponding with the head in the pressure regulator, will be approximately constant

up to the first plug ; this gives rise to a sudden decrease behind it. If there is no Mariotte flask, the pressure is increased by reason of the capillary constrictions and the gaseous friction until a value is reached corresponding with the friction of the whole system up to the last capillaries of the soda-lime tube. The Mariotte flask, which Pregl rightly termed a safety precaution, is used to avoid this excessive pressure and the possibility of leaks ; thus, atmospheric pressure exists at the most critical part of the apparatus, *i.e.*, between the plug and the first capillary constriction of the calcium chloride tube.

Numerous blank tests have shown that carbon dioxide and water are drawn into or expelled through a warm rubber connexion, according as the pressure in the apparatus is less or greater than atmospheric, respectively. The experiments also showed that the selective absorption of carbon dioxide by rubber, and its liberation again in the direction of lower concentration, is, at most, merely reduced by impregnation with vaselin (p. 39), whereas the normal capacity of rubber to absorb and liberate water vapour is almost entirely removed by such treatment. It follows that any carbon dioxide which penetrates into the rubber connecting tube during the short period of the combustion is almost quantitatively removed by the following stream of air free from carbon dioxide. From this standpoint pressure-equalisation by the Mariotte flask appears to be entirely unnecessary. Even impregnated rubber tubing, however, often suddenly and unexpectedly develops faults due either to capillary cracks which have developed in the course of time, or to the actual porosity of the tubing. The tubing between the combustion tube and the calcium chloride tube is more exposed to injury than that between the two absorption tubes, on account of its greater temperature.

To sum up, the Mariotte flask is not essential if the rubber connexions are faultless. In spite of this it is strongly recommended because it serves :—

1. For the calibration of the tube (p. 52).
2. For slow aspiration of air through the freshly filled absorption tubes.
3. For testing for leaks in the apparatus.
4. For checking the gas velocity during the combustion.
5. For measuring the 100 ml. of air required for the quantitative replacement of gas.
6. To ensure accurate absorption of the products of combustion.

As shown in Figs. 30 and 39, the Mariotte flask consists of a bottle (capacity, 1–2 l.) near the base of which is inserted a bent glass tube (width 4, bore 2 mm.) in such a manner that it can be rotated in a vertical plane and forms a one-armed syphon. The glass tube is bent at right angles at each end, the outer angles being perpendicular to one another, and it should be at least as long as the bottle is high. A rubber stopper is unsuitable, because it holds the glass tube too stiffly ; a cork stopper enables any position to be maintained. In the

neck of the flask is a doubly-bored rubber stopper, one hole of which is closed with a glass rod or stopcock, which is removed or opened when the apparatus is not in use to prevent water being drawn out if the temperature rises. Through the other hole is a glass tube of 3–4 mm. bore, bent twice at right angles and reaching to the bottom of the bottle. Its free end is connected with a small calcium chloride tube (Fig. 38) by means of ordinary rubber tubing. The latter is connected with the soda-lime tube during the analysis by means of 30–40 mm. of the treated tubing. The Mariotte flask should stand on a tripod with side supports (Fig. 30).

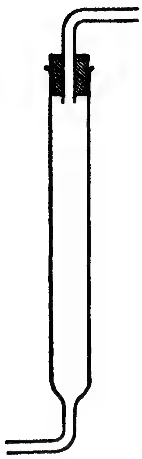

FIG. 38. Calcium chloride tube with connecting tubes. Actual size.

Unterzaucher overcomes the necessity to replace the syphon in a vertical position at the beginning of every determination (to avoid leakage during weighing) by inserting a stopcock (a, Fig. 39) in the inlet tube of the flask. The closure of this prevents suction of air and the accompanying leakage of water when the syphon is lowered, and it is possible to leave the position of the syphon unchanged while the absorption apparatus is being filled; afterwards, the original conditions of temperature and pressure may be restored by simply re-opening a. With substances very rich in hydrogen, alterations in air resistance may occur after the calcium chloride filling has been in use for some time; a slight alteration of the height of the syphon at once remedies this.

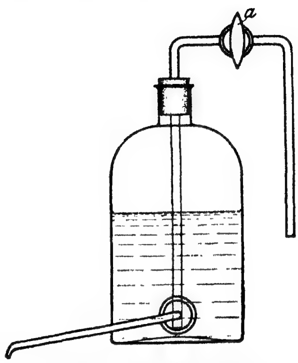

FIG. 39. Unterzaucher's modified Mariotte flask.

Reagents

Sodium Hydroxide. A 5% solution is used for filling the pressure regulator. For one filling, 40 gm. in 800 ml. of water are taken.

Potassium Hydroxide for the bubble-counter; see p. 35.

Asbestos. Commercial Gooch crucible asbestos is purified as follows: The coarse fibres and powder are separated mechanically, and the residue is washed on a coarse suction-filter with distilled water. It is then transferred to a large platinum or glass dish, digested for 5 hrs. with concentrated hydrochloric acid, and washed with hot distilled water on the filter until the filtrate shows no traces of chloride (see p. 93); most of the water is then removed from the purified asbestos by slowly drawing alcohol through it. The asbestos is placed in a crystallising dish, loosened with forceps, and left to dry for several hours in a desiccator over phosphorus pentoxide. The last traces of moisture are removed in the drying-oven at 120° C., and the pure white

asbestos is stored in a wide-necked bottle. Asbestos purified in this manner neither retains water nor temporarily absorbs carbon dioxide; it is not in any way a source of error.

Silver Wire or **Silver Wool** can be purchased in the pure state, but it is advisable to reduce the silver wire before use in a current of hydrogen in a glass tube, and afterwards to ignite it in a stream of oxygen. The same procedure is applied to the silver fillings of used tubes which have become charged with halogens and sulphur, in order to regenerate them for further use.

The Mixture of Lead Chromate and Copper Oxide is made by impregnating pieces of copper oxide (in wire-form, 4–5 mm. long) with an equal weight of finely powdered commercial lead chromate in a nickel-steel dish over a strong blowpipe flame. The mixture is stirred very thoroughly with an iron rod so that the whole surface of the copper oxide is coated compactly with the molten chromate, and the alkali which always adheres in small amounts to copper oxide is converted into chromate. Hennig [17] prefers a mixture of copper oxide pumice and lead chromate pumice (*cf.* p. 62).

Lead Peroxide is the most critical of the reagents and, therefore, has been exhaustively investigated. With substances consisting only of carbon, hydrogen and oxygen, only an oxidising tube-filling is needed. If, however, nitrogen is present, of the two reagents available for fixing oxides of nitrogen (namely, metallic copper and lead peroxide) copper must be excluded, because it is very readily oxidised in presence of excess of oxygen. Lead peroxide, therefore, must be used, despite its property of taking up moisture temporarily and of absorbing carbon dioxide in the dry state and ultimately giving it up in presence of small amounts of water. At 180°–200° C., however, the adsorbed water is quickly given up again, and the stage of drying reached is not high enough for the adsorption of carbon dioxide to occur.[1] In order to maintain the correct conditions, Pregl's hollow mortar should be used to obtain a constant value for the vapour pressure of the water-lead peroxide system, and it is absolutely necessary that the lead peroxide should be prepared as follows :—

Commercial lead peroxide (150 gm.) is digested with concentrated nitric acid (density, 1·4) for 2 hrs. on the water-bath. After standing for 1–2 hrs. it is washed repeatedly by decantation with distilled water, with stirring, until nitric acid can no longer be detected in the wash-water by the diphenylamine-sulphuric acid test. The slimy residue is then almost completely dried on the water-bath, and cut into small 2-mm. cubes with a spatula. The pieces are rotated in a roomy wide-necked bottle, either by hand or by a slowly rotating lathe, so that they polish one another; they are then sieved. After re-moistening and drying, the powdery residue can again be cut up into cubes and treated as described. The final preparation should be black (not brownish) in colour, and after 6 hrs. of ignition in the combustion tube, excellent hydrogen values can be obtained with it. It is not too quickly exhausted

in combustions of substances containing nitrogen, and it does not swell unduly in heating.

Krönig Cement. A molten mixture of 1 part of white wax with 4 parts of colophony is poured into cylindrical moulds.

Dekalin (decahydronaphthalene, b.p. 188° C.) or **Cymene** (b.p. 176° C.) for the heating mortar. The former is usually preferred. Commercial dekalin can be used much longer if it is first shaken two or three times with concentrated sulphuric acid, washed with water, dried carefully and distilled. The first light oil, which contains small amounts of acid, is rejected, and the middle fraction (b.p. 186°–190° C.) is collected directly in the stock bottle ; it is much less inclined to resinify than is the original dekalin.

Calcium Chloride. Groat size (fused) is used. Merck's preparation loses 1·2% on ignition. If used with soda-lime, as the absorption reagent for carbon dioxide, good results may be expected for hydrogen. Alternatively **Anhydrone** (magnesium perchlorate) can be used directly in the absorption apparatus, whilst Reihlen [14] prefers a layer each, in order, of anhydrone and phosphorus pentoxide.

Absorbent for Carbon Dioxide. The soda-lime used must have the correct moisture content (see p. 50), but as no quantitative specification can be given at present the beginner must usually find out for himself the right conditions, often as the result of some incorrect analyses. Every new delivery of soda-lime is ignited in order to ascertain the necessary water content. For this, an ignited 8-ml. porcelain crucible is weighed in a weighing-bottle on a macro-balance. Then 4–5 gm. of soda-lime are weighed into it, and the crucible is ignited over a gas flame at dark-red heat for 10 mins. and cooled in a desiccator over phosphorus pentoxide. It is then re-weighed in the closed weighing-bottle, and the loss on ignition so determined.

Reliable values for carbon are obtained if commercial soda-lime is moistened with as much water as will add 33 parts of water to 100 parts of the ignited anhydrous soda-lime. In order to prepare a large amount of soda-lime, a wide porcelain dish containing 50 gm. of soda-lime, and a crystallising dish containing 7·0 ml. of water are placed in a large desiccator. After 2–3 days the soda-lime will have absorbed all the water. If the desiccator is evacuated quickly to about 5 mm. of mercury and left closed, the water will be absorbed in 1–2 days. Alternatively, place a weighed amount of soda-lime on a filter paper, and spray it with rather more than the correct amount of water (because the filter paper absorbs some) ; roll the soda-lime about on the filter paper, to moisten it uniformly, and place it at once in the absorption tube. The necessity to moisten the soda-lime is usually explained by the fact that dry soda-lime does not absorb carbon dioxide ; it is more likely, however, that it serves the purpose only of preventing the removal of the last traces of moisture from the gases passing over it.

An excellent absorption agent for carbon dioxide is now sold, under the name of Ascarite. It consists essentially of shredded

asbestos, impregnated with 100% sodium hydroxide solution, which is heated at 170° C. for about 4 hrs. and cooled and ground. This product absorbs on an average about 500 mgm. of carbon dioxide per filling, that is, three to four times as much as soda-lime. Moreover, the progress of absorption is indicated by a marked coloration of the absorbing agent, so that it is always possible to see when the filling is becoming exhausted. The exhausted filling is, however, not so easily removed from the tube as is soda-lime, and absorption is always accompanied by consolidation and an increase in volume. The spent filling must, therefore, first be soaked overnight in slightly acidified water, although the difficulty may be overcome to a great degree by using the Ascarite mixed with soda-lime, or with glass wool. It is also to be noted that, compared with soda-lime, the vapour-tension of the water in Ascarite is extremely small. It is consequently necessary to remove the last traces of water from the calcium chloride used in the U-tube of the bubble-counter, in the calcium chloride tube, and in the Ascarite tube, by heating over a luminous flame in a round-bottomed flask evacuated by a water pump. Otherwise the water-vapour from the calcium chloride tube will be absorbed by the Ascarite in the soda-lime tube, and give a high result.

Oxygen and Air. Nowadays oxygen is usually obtained directly from steel vessels containing liquid air, or from constant-pressure gasholders filled with such oxygen (*e.g.*, those evolved by Hohl[18] and by Lindner[19]). The former method is preferred, because a 3-litre container in daily use lasts more than a year, the same delivery of gas is always being used, and the cost is less. The possibility of small amounts of organic matter in the gas from liquid oxygen (derived from the oil used in the compressors) should, however, not be overlooked.

The air, about 80 ml. of which are drawn through the apparatus and absorption tubes for each analysis, must be completely free from carbon dioxide and organic constituents. For removal of carbon dioxide, the U-tube on the bubble-counter usually suffices. Organic vapours contained in the air of the laboratory are avoided by filling the gasholder in the open air, or by drawing in pure air from outside. If, however, gasholders stand for some time in the laboratory, vapours of organic solvents may be absorbed by the sealing liquid and so may enter the gasholder. This source of error, though small, can be avoided if the air is used directly from a vessel, such as a cylinder fitted with a needle reducing-valve for regulating the pressure, and containing air at 160–180 atm. pressure.

A blank test must be made on new supplies of the gases; the calcium chloride tube should increase in weight by less than 0·05 mgm. and the soda-lime tube by not more than 0·02 mgm. If these limits are exceeded the gases must be free from vapours containing carbon and hydrogen, *e.g.*, by the "catalyser-pipe" (Fig. 40) of Böck and Beaucourt,[10] which is inserted between the three-way cock and the bubble-counter. This consists of a glass tube filled with platinised

asbestos, which is electrically heated at 600° C. Following the heating unit (H) is a coil, which cools the gases to room temperature before they pass through the next rubber connexion. The water and carbon dioxide so formed are retained in the U-tube which follows. The catalyser-pipe should be used only when the blank test on the gases indicates an increase in weight; it should not be included merely as a precaution, since the apparatus thereby only becomes more complicated and the extra connexions may give rise to additional errors.

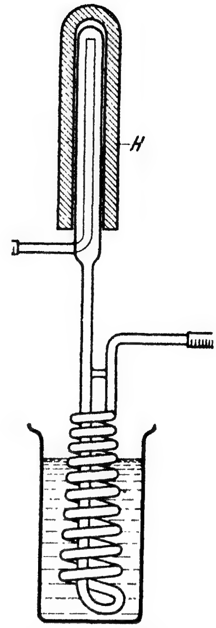

FIG. 40. Catalyser-pipe, with electrical heating unit (H).

Procedure

Setting-up the Apparatus. Any well-lighted laboratory bench may be used. Near the right-hand end should be 40–50 cm. of free space for setting-up the two gas containers, so that the gases may not have to travel too far to the pressure regulator. If space is restricted these may be placed on a stand under the working table.

The pressure regulators are cleaned successively with chromic acid, water, and distilled water, and are about three-quarters filled with 5% sodium hydroxide solution (p. 43); the bell-jars are inserted and connected with the gasholders by means of the matured rubber tubing, provided with precision pinchcocks (Fig. 31). If the gases are used directly from the steel containers, the pinchcocks are unnecessary, and connexion with the valves is made through two clean glass tubes, which touch the inlet tubes of the pressure regulators. They are connected with the valves by rubber tubing, the length of which is such that the inlet-tubes of the pressure regulators (which are normally immersed to a depth of 7 cm.) can be raised by about 2 cm. without stretching the tubing. The delivery tubes of the pressure regulators are connected to the three-way cock, which is greased with a little vaselin.

The electric combustion surface must be next adjusted by means of the resistance to a temperature of $550 \pm 20°$ C., a thermo-element being used to check that the casing of the furnace is uniformly heated. Thus, the thermo-element is placed exactly in the middle of the furnace, and the rheostat position corresponding with 550° C. is carefully marked. Subsequently the resistance is not inserted until the heater begins to glow. Finally, the temperature is checked at about 2 cm. from each end of the furnace.

Filling the Combustion Tube (Fig. 33). The tube is washed successively with hot chromic acid, distilled water and alcohol, and dried by warming it and drawing air through it with a water-pump.

The choking-plug necessary to produce the requisite gaseous friction is made by igniting purified asbestos (p. 43) for about 10 mins. on a platinum lid with the non-luminous flame of a Bunsen burner. On its compactness depends the height of the effective column of water in the pressure regulators, so that the gases may pass through the system at 4 ml. per min. It is important that no incompletely burnt substance reaches the absorption apparatus, on account of a sudden expansion of gas.

Small wads of the ignited asbestos are introduced into the tube with the platinum-tipped forceps, and pushed up to the point of attachment of the neck, with a glass rod (diameter, 5 mm.) with sharp edges; they are pressed against the wall of the tube, and packed carefully together (length, 6–7 mm.). The combustion tube is then attached to the bubble-counter and it is ascertained whether, at a pressure of 40–60 mm. of water in the pressure regulator, about 3–4 ml. of gas pass through the tube per minute (*cf.* p. 52). The somewhat larger resistance due to the subsequent filling of the tube is compensated for by a head of 10–20 mm. in the bell tube.

Lead peroxide is then poured on to the choking plug so as to form a column of height 20–25 mm., and is distributed by carefully tapping; any adhering to the wall of the tube must be removed by means of a plug of cotton-wool attached to a wire. A loosely packed 2-mm. asbestos layer is then inserted, followed by the silver filling (length, about 40 mm.). Its actual length depends on the mortars; thus, for a distance of 10 mm. between the mortar and the tube burner, 5–10 mm. of the silver filling should be in the wire gauze roll of the burner. With electric heating, however, the mortar is preferably only 5 mm. from the furnace, and the silver wool extends to the heating coil. A 2-mm. layer of asbestos is inserted next to the reduced silver, the tube is filled up with the mixture of lead chromate and copper oxide (a length of about 140 mm., in the case of a gas furnace); finally, a stopper of silver wool (about 15–20 mm. long) keeps the filling in place.

The long heater for electric furnaces is on wheels, so that it may be moved relatively to the moveable burner, *e.g.*, to heat the colder parts of the tube after the combustion and so to remove residual substances. The high heat conductivity of silver can be turned to account by using a 30-mm. silver layer which reaches the layer of copper oxide and lead chromate, the length of which is chosen so that the silver filling projects 5 mm. beyond the portion of the tube heated by the long heater. If, towards the end of the combustion, the moveable burner is moved up to the long heater, then heating the end of the silver layer for a minute suffices to ensure that the last fragments of the substance near the long heater are burned. It is then unnecessary to move the long heater. Two rolls of iron wire gauze which do not fit too closely (diameter about 11 mm.) are placed round the portions of the tube heated by the long heater and burner, as shown in Fig. 33; the longer roll is unnecessary with electric furnaces. The tube is then wrapped

in a strip of asbestos paper, over the layer of silver adjoining the lead peroxide (to obviate air currents through the mortar), and placed in the mortar, which should contain sufficient dekalin to reach to a height of 10–20 mm. in the vertical tube during boiling. The mortar should be not more than 1 cm. from the neighbouring wall of the combustion stand. The mouth of the tube is closed with a well-fitting cork with fine pores, and the side-tube is connected with the U-tube by means of matured rubber tubing.

A well-filled tube can be used for at least 200 analyses, if it is not unduly heated and if the layer of lead peroxide is never exposed to a temperature higher than 200° C. If substances containing halogens or sulphur are burnt consecutively, the silver wool adjacent to the boat should be renewed after 10–20 analyses.

The method of filling described may appear meticulous, but it is the result of much consideration and many experiments. The lead peroxide normally ensures the retention of the higher oxides of nitrogen probably formed from nitro-, nitroso-, and hydroxyazo-compounds, but if halogens or sulphur are present it is not always adequate, particularly if it already contains lead nitrate. The best reagent for the absorption of halogens is hot metallic silver, and lead chromate is suitable for retaining the oxides of sulphur. By placing these two reagents in front of the lead peroxide, the burden on it is relieved and its only function (that of absorbing the higher oxides of nitrogen) remains unimpaired. J. B. and V. Niederl [20] find that in many cases the lead chromate is unnecessary if the silver is maintained at above 400° C., as it is then able to absorb the oxides quantitatively. Belcher [21] favours a modification of Friedrich's method (described more fully on p. 61) in which interfering gases are absorbed in lead peroxide at 180°–200° C. ; the reagent is contained in two small boats, instead of being packed into the tube, and may, therefore, easily be renewed after every few analyses. Niederl and Whitman [22] prefer to use a reduced copper spiral (which is heated in a stream of nitrogen to prevent oxidation) to decompose oxides of nitrogen ; an advantage claimed is the very low blank. The use of cerium dioxide on pumice instead of copper oxide is also advocated by some workers.[21, 23]

Other and more far-reaching suggestions for the modification or simplification of the Pregl combustion tube have been made from time to time (*cf.* p. 60), but so far as the beginner at any rate is concerned, they should be approached cautiously. Such methods may prove quite satisfactory in the hands of their originators, but unless they have been described with the attention to detail necessary for work of this kind, unexpected pitfalls may arise. The Pregl method is, therefore, to be preferred by reason of the great amount of attention and experiment it has undergone.

Filling the Absorption Tube. New apparatus is tested (see p. 57) and placed for an hour in warm dilute hydrochloric acid to obviate the loss in weight on wiping (due to loss of material from the surface, see

below); it is then washed successively with distilled water and alcohol, and dried at 110° C.

Filling the Calcium Chloride Tube (Figs. 36, *a* and *b*). To prevent the scattering of dust, a small loose pad of cotton-wool is placed on the diaphragm of the ante-chamber. On this are placed either two or three pieces of coarse, porous calcium chloride or, if only the groat size available, two small capillary tubes (length, 10–15; diameter, 1·5–2·0 mm.) and a layer of about 20 mm. of calcium chloride. On this is placed a small pad of cotton-wool, 2–3 mm. thick, and groat-sized calcium chloride is added up to the ground-in joint. The calcium chloride is covered with a large pad of cotton-wool, and the joint is cleaned with cotton-wool twisted round a coarse steel wire. The ground joint and stopper are now warmed gently, in a small, just non-luminous flame, some Krönig glass cement (p. 45) is placed on the stopper, and the warm stopper is screwed into the ground joint. The excess of cement is removed after cooling, mostly mechanically, and finally by a rag moistened with benzene. Since calcium chloride absorbs small amounts of carbon dioxide, the tube is therefore attached, by the end opposite to the joint, to a Kipp carbon dioxide generator. The air is first displaced by carbon dioxide passing in the same direction as in the actual analyses, and the open end is then closed and the apparatus left for 10 mins. under pressure. The carbon dioxide is then removed by sucking 100 ml. of air through by means of the Mariotte flask. If Anhydrone is used this stage may be omitted; moreover, Anhydrone has the advantage that it will absorb about 200 mgm. of water, whilst calcium chloride begins to cake even after absorbing 80–100 mgm., and must be renewed.

The Soda-Lime Tube (Fig. 36, *b*) is filled in the same way. A plug of cotton-wool about 5–6 mm. long is first inserted, and on this placed a 30-mm. layer of groat-sized calcium chloride or Anhydrone. A layer of cotton-wool, 2–3 mm. thick, forms the boundary between the calcium chloride and the soda-lime which follows, and prevents the calcium chloride from rolling out when the soda-lime is renewed. The moistened soda-lime (p. 45) is now filled in up to the ground-in joint, covered with a cotton-wool pad, and the stopper cemented in and cleaned. Oxygen is passed through the tube for 30 mins., followed by 100 ml. of air; the taps are closed for 5 mins. so that there is a slight pressure inside, opened for 5–10 secs., and then closed before weighing.

One filling absorbs 120–140 mgm. of carbon dioxide. Before an analysis, the absorption tubes are closed by caps of rubber tubing and kept near the balance on a metal stand, which supports them at two points only. Finally the calcium chloride tube (Fig. 36, *a*) connected with the Mariotte flask is filled; this filling should be renewed monthly. Tubes filled in this way with calcium chloride weigh about 6 gm.; with soda-lime, about 7 gm.; and with Ascarite, about 9 gm.

Before making the first analysis it is advisable to practise cleaning and wiping the absorption apparatus so as to check the constancy of weight.

For cleaning the connecting tube, a steel wire (diameter, 0·75 mm.), with cotton-wool wound round its roughened end, is used. For cleaning and wiping the external surfaces, two flannel cloths and four chamois leathers (all 6 × 10 cm.) are used. They are kept in three roomy glass jars, and when not in use are protected from dust by inverting the jars. The leathers must be perfectly clean; they are thoroughly washed with luke-warm soapy water containing a few drops of ammonia, and then dried on a string at room temperature. Before use, the flannel cloths are placed in distilled water, then wrung out and rolled in a clean, dry towel, and most of the water is removed by squeezing hard. The moist cloths are then placed in a glass box and left in it, with the leathers, for an hour. On very humid summer days this precaution may be omitted.

The caps of the apparatus are removed, and the tubes are placed on a soft support. The calcium chloride tube is held in a moist cloth, and the connecting tubes are cleaned as far as the constrictions with the cotton-wool, care being taken that any particles of rubber are removed. The external surfaces are cleaned by holding a flannel cloth in each hand and wiping the tube twice from the middle over the connecting tubes to the ends, and rotating the apparatus. It is then wiped similarly with the first pair of chamois leathers, and then with the second, and replaced on the metal stand. The soda-lime tube is cleaned similarly.

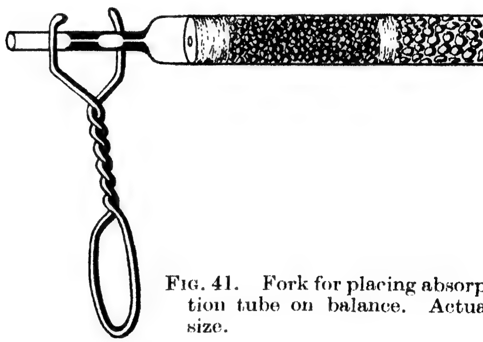

FIG. 41. Fork for placing absorption tube on balance. Actual size.

The wiped apparatus must not be handled. Five minutes after removal from the stand or from the neck of the combustion tube, the calcium chloride tube is placed on the hook of the left balance suspension by means of an aluminium fork (Fig. 41), and the counterpoise is placed on the right pan. The final weighing is made 5 mins. after placing the tube in the balance (*i.e.*, 10 mins. after removal), so that the cleaning and weighing take exactly 15 mins. With practice the weight of the absorption apparatus is reproducible to within ± 0·005 mgm.

In cases of doubt Friedrich's method [15] of using a third absorption tube in a control experiment, so as to obtain an actual correction, can be followed. McNevin and Varner [24] have extended this principle with success, using the third tube as a tare as well as for a control. This tube is the same in design as the other absorption tubes, but it contains the drying agent in the first half, and Ascarite in the other; Drierite (anhydrous calcium sulphate) is sometimes favoured for the former, but it has no special advantage over Anhydrone. The tare

tube, should weigh slightly less than either of the other tubes. It is inserted in the combustion train after the carbon dioxide absorption tube, and treated similarly to the other tubes, except that it is placed on the right-hand pan of the balance when its fellow tube is being weighed on the left pan. Clean, white cotton gloves are used when handling the tubes, and wiping is confined to the use of the thumb and first finger round the outside of the tips of the capillaries, a pipe-stem cleaner being used for the inside. Under these conditions the tubes may be weighed without waiting, and Pyrex glass may be used in their construction. Royer, Norton and Sundberg [25] reached similar conclusions. Since, moreover, the constancy in weight is maintained for 1 hr., a rigid weighing programme becomes unnecessary.

In general, apparatus undergoes an apparent diminution in weight after handling and wiping, due principally to the warming of its surface, but after 15 mins. a constant value is reached if the necessary conditions are fulfilled. Apparatus occasionally acquires a maximum weight after wiping, and then becomes steadily lighter. Pregl and Brunner traced this to the use of too dry a chamois leather; this produces electrostatic charges on the glass surface. It is eliminated by keeping the chamois leathers in the glass bottle in which the moist flannel cloths are sometimes placed, for an hour.

According to Pregl, the absorption apparatus can be weighed accurately only if the balance room has a temperature equal to, or higher than, that of the combustion room. Thus, if the tube is brought into a very cold balance room, the contraction of the air in it draws in water vapour, and positive errors of over 0·07 mgm. result. To guard against this, in the case of the soda-lime tube, Pregl recommended placing a doubly folded piece of moistened flannel over it during the combustion, but this appears to be unnecessary with electrically heated apparatus.

Adjustment of the Gas-Current. For the quantitative conversion of organic substances into carbon dioxide and water, a certain period of contact between the gases evolved and the hot filling is necessary; numerous experiments have shown that this corresponds with a gas-speed of 4 ml. per min. under a 50- to 80-mm. head of water. The bubble-counter is used as a permanent check on the speed of the gas in the hot tube, as well as to standardise the rate of aspiration by the Mariotte flask after the insertion of the absorption apparatus. The standardisation is carried out, after igniting the tube for 6 hrs., as follows:

The pressure regulator is first set with a water-head of 50–70 mm., and the bubbles passing through the bubble-counter in 10 secs. are counted using a stop-watch. Then the calcium chloride tube of the Mariotte flask is connected with the neck of the combustion tube by means of rubber tubing, and the syphon of the Mariotte flask is so adjusted that the bubble-counter again shows the same bubble-frequency. The gas passing through the tube in 5 mins. is measured

by the water which flows away into a measuring cylinder placed under the syphon. The calculation of the speed of the current may be illustrated by an example, as follows :—

The first adjustment of the pressure regulator gave 18 ml. of water in 5 mins., for nine bubbles in 10 secs. ; thus, the gas-speed was only 3·6 ml. per min. The correct bubble-count, which is obtained by raising the head of water in the bell gasholder, is thus : volume required $\times$ bubble count of the test/volume found ; *i.e.*, $20 \times 9/18 = 10$ bubbles in 10 secs. This speed of 4 ml. per minute is checked, and then the second pressure regulator is adjusted similarly. These adjustments may have to be altered subsequently, owing to the warping of the tube or to displacement of the lead peroxide. An excessive suction is less objectionable than a positive pressure in the absorption train ; the latter may lead to losses of carbon dioxide and water, whereas, with properly impregnated rubber tubing, neither water nor organic vapours are lost as the result of an increased suction.

The result of these precautions is that the gases issue from the combustion tube with a definite velocity, which is easily established and determined empirically, so that the gas remains in contact with the filling for a definite period of time. This is ensured by the choking plug near the exit from the combustion tube. Its effect differs essentially from that of a pinchcock which, at best, only ensures the regularity of the gas stream, and not a uniform time of contact with the hot contents of the tube. However, a choking plug is effective only when the pressure is constant, and as this constancy is difficult to achieve with a pinchcock, the pressure regulator described is used.

Weighing in general, together with the preparation of the sample for this operation, is dealt with on p. 16, but a few points particular to this determination arise. Thus, very hygroscopic substances should be dried in the combustion tube itself, or if several analyses are to be made on one sample, it may be advisable to use undried samples, and to correct the results for the moisture content (Alber [26]). In other cases the sample is transferred from the weighing tube (which is re-stoppered immediately afterwards) to a platinum boat (*g*, Fig. 10, p. 18) or cylinder (*d*, Fig. 10, p. 18) in a large desiccator containing freshly dried phosphorus pentoxide ; the boat is not removed until the last minute before the analysis proceeds.

The constancy of the temperature during the weighing of absorption tubes is also important ; errors of $\pm$ 0·01 mgm. have been recorded for changes of 1° C. With 3 mgm. of a substance containing 70% of carbon, the error in the determined carbon-content due to the soda-lime tube is $\pm$ 0·08% ; with a 1-mgm. sample it may well exceed $\pm$ 0·2%, and may even attain 0·4%.

Preparation for the Combustion. It is advisable to adhere closely to the following systematic procedure, which is the result of experience gained in many analyses, and in which the sequence of individual operations is arranged suitably.

The doors of the balance are first opened for "acclimatisation." The closing cap is then removed from the neck of the combustion tube, the supply of oxygen is turned on, and the three-way tap is set for the passage of the oxygen. The mortar is heated with a large regulated flame so as to boil the heating liquid quickly, and the tube filling is heated, the resistance being adjusted as described on p. 39. The three-way cock is then turned through 45°, to prevent the escape of oxygen from the gasholder of the pressure regulator when the cork is removed from the combustion tube. The mouth of the tube is cleaned with a wad of cotton-wool wrapped tightly round a steel wire, the cork is again inserted, and the three-way cock is returned to its original position. The tube is then heated at about 4 cm. from the side-tube; the roll of wire gauze protects against overheating. At the same time the flannel cloths are moistened and placed in the glass vessel with the chamois leathers, and covered (p. 52).

When the mortar liquid has boiled and the tube is at the correct temperature (p. 39), then the air-supply is turned on, and the bubble-counter (set for 4 ml. per min.) is checked, both for air and oxygen. The air-supply is shut off and the three-way cock is opened, so that the tube is filled with oxygen while the sample is being weighed out.

Every series of analyses is prefaced by a determination made on a test substance (*e.g.*, alizarin, salicylic acid, cholesterol, etc.). This is weighed out, placed on the copper block of the small desiccator, and brought to the apparatus. The absorption train is weighed as described on p. 51. The Mariotte flask is filled, the treated rubber tubing is moistened with a little glycerin by means of cotton-wool wrapped round a steel wire, and the absorption tubes are connected by a glass-to-glass joint. The sample is then placed in the combustion tube, a shorter piece of rubber tubing is drawn over the side-tube of the calcium chloride tube, to the middle of it, and connexion with the neck of the combustion tube is made with a glass-to-glass joint; the calcium chloride tube is also attached by rubber tubing to the Mariotte flask, the syphon of which must be upright. If the apparatus is completely gas-tight, no bubbles rise in the bubble-counter.

The current of oxygen is now cut off from the bubble-counter, the cork is removed from the mouth of the combustion tube, and the boat containing the substance is pushed into the tube by lifting the copper block in the left hand so that it touches the lower edge of the tube, and then sliding the boat horizontally into the tube by means of previously ignited platinum-tipped forceps. A clean glass rod with rounded edges is used to push it in 40–50 mm. from the layer of silver wool, but no nearer in the case of volatile substances which melt at room temperature. This is because, even with electric heating, the tube at 30–35 mm. from the ignited filling is 5°–8° C. hotter than at 45 mm., and before actual combustion begins the substance may sublime backwards. Substances of low melting-point should be at least 60–70 mm. from the tube filling. Very volatile solids are contained in a weighed and sealed capillary

(p. 18), which is placed in a cylinder of platinum foil 4–5 mm. long; the sealed tip and the mid-point of the handle are then broken, and the platinum cylinder is placed in the combustion tube in such a way that, as the closed end becomes hot, the sample is expelled at the tip, which should not project outside. The tube is now closed with the cork, and the short roll of wire gauze is placed so that its front edge reaches just to the handle of the boat. At this stage it is advisable to test for leakages again (see above).

The bell gasholder is now filled with oxygen, and connected with the apparatus by turning the three-way cock. The syphon of the Mariotte flask is lowered until the bubble-counter shows the same bubble frequency as before the inclusion of the absorption train, and it should in no case be moved before the end of the analysis. The copper heater of the hollow mortar is placed over the first capillary constriction of the calcium chloride tube; or with substances having very high hydrogen contents, over both capillaries.

The Combustion. The short roll of wire gauze is heated with the full, just non-luminous flame of the moveable burner; a fall in bubble-frequency occurs, but after a short time this returns to the original value. After a few minutes, when the substance has melted, sublimed, distilled, or even partly carbonised, the gauze roll is advanced some millimetres, and after 10–20 secs. the burner is moved after it to about the same extent. Every time that the burner is moved forward the bubble-frequency falls, and it is therefore necessary to leave the burner stationary long enough to restore the original value. If the burner is advanced too quickly, rather large amounts of gas may be formed suddenly, and the pressure set up is then transmitted against the direction of the gas-current up to the bubble-counter. This usually leads to serious losses, because the vapour of the substance analysed passes behind the heated zone of the moveable burner, or even into the neighbourhood of the opening of the tube. In this connexion particular attention should be paid to substances which form liquid drops in front of the long burner. Substances which deposit charcoal and are normally very difficult to burn, may be burnt without difficulty in platinum boats, if the burner is removed for a short time and the cooled boat is subsequently again heated; the particles of charcoal then burn with a shower of sparks.

Attention can be given here only to those precautions which must be strictly observed by the beginner. From the first stages, the expert knows exactly how the combustion should best be regulated, and if he has to analyse the most varied substances in succession, he can, as a result of experience burn each substance in the best way. For this reason devices for the automatic regulation of combustion (*e.g.*, that of Reihlen,[14] in which the speed is controlled by the pressure of the oxygen) must be used cautiously and only by the experienced, since the setting depends rather critically on the weight of substance used

and on its degree of volatility. Normally, 10 mins. suffice for the combustion with oxygen.

If the moveable burner has been moved up to the tube burner, the valve of the air supply is opened, and the three-way cock is turned through 180° to admit air. A 100-ml. measuring cylinder is substituted for the beaker under the syphon, and the air sent through the system is measured by the water collected in the cylinder. The tube is now heated to redness in less than 10 mins. with the moveable burner, starting at about 70 mm. from the cork, and during this time the boat for the next analysis may be boiled out and ignited. If much condensation of water occurs in the capillary of the calcium chloride tube, obstruction may occur, and with substances very rich in hydrogen the water is driven over into the ante-chamber by touching the capillary with nickel forceps heated in the flame of the moveable burner. If this is ineffective, the burner is pushed back a few centimetres, but not so far as to drive alkali into the horizontal tube of the bubble-counter. The water is then usually driven out of the capillary in a few seconds by the extra pressure. The same procedure is used, if necessary, for the calcium chloride tube. By the end of this second ignition, 40 ml. of water should have been collected in the measuring cylinder. The next 15 mins., corresponding with the collection of a further 60 ml. of water, are used for weighing the next sample.

When 100 ml. of air have passed through, the copper heater is removed from the calcium chloride tube, the syphon of the Mariotte flask is raised upright, and the rubber connexion is removed from the end of the soda-lime tube, the tube being held in the right hand. The combustion tube is then disconnected at the neck, and the rubber tubing placed near the apparatus. Finally, the rubber tubing is removed from the calcium chloride and soda-lime tubes.

The absorption tubes are cleaned and wiped (p. 51), and the calcium chloride tube is weighed first, 10 mins. after removal from the apparatus. The absorption tubes are placed on the metal stand, the boat is removed from the tube with a platinum hook fixed in a glass rod ; and a lens is used to ascertain if there is any residual ash to be weighed. The mouth of the tube is closed, the air supply is shut off, and oxygen is again passed through the tube. After weighing, the absorption tubes are ready for the next determination.

Normally, 45–60 mins. elapse between the beginning of combustion and the final weighing, but in cases where speed is a first consideration it is possible to reduce this to 25–35 mins. for samples up to 50 mgm. in weight by packing the filling tightly and using a longer tube (Brodie,[27] Titov [28]) ; by increasing the rate of gas-flow to 5–6 ml. per min. (Lindenfeld [23]) or even to 35 ml. per min. (Belcher and Spooner [29]) ; or by working at a higher temperature. In order to prevent back-diffusion of unburnt gases under these conditions Lindenfeld inserts a platinum contact, which is heated during the combustion, about 4 cm. behind the boat containing the sample.

Calculation

Since, $\%H = \text{wt. of } H_2O \times 2{\cdot}016 \times 100/\text{wt. of sample} \times 18{\cdot}016$; and, $\%C = \text{wt. of } CO_2 \times 12{\cdot}000 \times 100/\text{wt. of sample} \times 44{\cdot}000$.
Therefore,
$\log(\%H) = \log(\text{wt. of } H_2O) + \log(2{\cdot}016/18{\cdot}016) + 2 - \log(\text{wt. of sample})$;
and,
$\log(\%C) = \log(\text{wt. of } CO_2) + \log(12{\cdot}000/44{\cdot}000) + 2 - \log(\text{wt. of sample})$.

An example follows; the beginner should copy it into his notebook.

Alizarin ($C_{14}H_8O_4$)	Calcium chloride tube	Soda-lime tube	Logarithms			%H	%C
			H	CO_2			
			97313	91381	Theory	3·36	69·98
3·318	49·00	20·25	04884	43573			
0·123	48·06	12·05	49553	49553	Found	3·29	69·99
3·195	0·94	8·20	51750	84507	Error	−0·07%	+0·01%

Accuracy and Sources of Error

Blank tests are necessary to ensure that the apparatus is working satisfactorily, and to detect sources of error appearing during the combustion. They are carried out in exactly the same way as the combustion, except that no substance is inserted in the tube; if they are made correctly, errors due to gases, rubber tubing, tube filling, or absorption agents are indicated by increases in weight exceeding 0·05 mgm. for the calcium chloride tube and 0·02 mgm. for the soda-lime tube.

If we first deal only with possible errors up to the absorption tubes, then we have to consider especially air and oxygen which derive organic vapours from their containers or absorb them on the way to the combustion tube (see p. 46). Impurities do not usually occur in gases from steel cylinders because, owing to the risk of explosion, the valve is sealed with water only; for the same reasons the needle-valve must not be greased or oiled. If, nevertheless, the gases are suspect, then a blank test is made using air only. If this results in no increase in weight, then oxygen is led through three or four times, when the absorption tubes will probably increase in weight. The gases can also take up adsorbed organic constituents from rubber tubing as they pass through it, and to detect this source of error with certainty tubing of various lengths is tested. In most cases such errors are eliminated by steaming out the tubing for 1 hr. If satisfactory gases cannot be obtained, the catalyser tube (p. 47, Fig. 40) is introduced before the bubble-counter.

In the further part of the apparatus (excluding the U-tube, which is discussed on p. 35) up to the combustion tube, there are practically no sources of error if glass is in contact with glass throughout.

Errors from the filling of the combustion tube are usually due to the lead peroxide. Thus, it is found occasionally that a freshly filled tube or a tube that has not been used for some time gives up small amounts of water after ignition for 6 hrs. The remedy is longer ignition. Increases in the weights of both absorption tubes, particularly the soda-lime tube, are due to lead peroxide which has not been prepared according to the instructions and gradually gives up the traces of nitric acid produced during its manufacture. After 150–200 analyses, exhausted lead peroxide may cause considerable errors, resulting in large or small fluctuations in the weights of both absorption tubes. In this case, the combustion and absorption tubes must all be refilled.

Increases in weight of both absorption tubes often occur in blank tests if faulty rubber tubing is used, particularly between the combustion tube and the calcium chloride tube. Moreover, the completely matured rubber tubing may absorb moisture from the air and give it up again on heating. When, therefore, the humidity of the air is rather high, the tubing is kept in a desiccator containing calcium chloride, without evacuation. Positive errors, particularly in the weight of the calcium chloride tube, are also due to the use of too much glycerin.

Negative blank values may be due to the differences in the activities of the absorption agents in the different parts of the apparatus. Calcium chloride is seldom a source of error in freshly filled absorption tubes, but if soda-lime is too dry it removes the last traces of moisture from the gases passing over it. The remedy is indicated on p. 45. The moisture-equilibrium of the calcium chloride may be upset if the U-tube becomes relatively damp after long use, and gives up water to the calcium chloride tube in the blank test ; newly filled soda-lime tubes also tend to increase in weight. The remedy is to refill the U-tube, and the calcium chloride and soda-lime tubes. It is advisable when refilling the calcuim chloride tube to charge the U-tube and the soda-lime tube with the same preparation of calcium chloride or Anhydrone, and if the apparatus has been idle for some weeks, all absorption agents should be renewed. After longer interruptions the tube should be ignited in a current of oxygen for at least 3 hrs. before re-use.

Other sources of error are not revealed by blank tests. Thus, a spent filling of soda-lime always leads to low carbon values, and spent lead peroxide can lead to false analytical results before its condition is detected by the blank test, or even by a test analysis with a known substance containing carbon, hydrogen and oxygen only. After prolonged use neither lead peroxide nor silver retains quantitatively all the oxides of nitrogen produced in the combustion of organic substances containing nitrogen or halogens. It is possible to guard against this by following the blank determination with a test analysis on a known substance containing nitrogen or halogen. It is particularly important to check combustion tubes which have been used for some 100 analyses of substances containing halogen or nitrogen, and in which further samples of this kind may be analysed.

Besides the well-known phenomenon of the temporary absorption of water, lead peroxide loses water if it is heated in a current of dry air or oxygen for 2–3 hrs. at the temperature of boiling dekalin; during the first analyses, therefore, the lead peroxide readsorbs the water lost, so producing a low value for hydrogen. The came criticism applies to the use of phosphorus pentoxide as an absorption agent,[11] and even with calcium chloride or Anhydrone slightly low values for hydrogen (though still within the limits of permissible error) have been obtained in an analysis which was separated from previous analyses by an interval of 1·5 hrs. during which the tube was heated in a stream of oxygen. For this reason it is most desirable to carry out a preliminary normal combustion with an unweighed substance (*e.g.*, 5–7 mgm. of alizarin) and in unweighed absorption apparatus, in a tube which is heated for 1–2 hrs. (so as to establish equilibrium throughout the whole system) before making the first test analysis.

On the whole, an average operator should be able to attain results having a variation of 3 parts per 1,000; however, Power [30] considers that a skilful worker should attain 2 parts per 1,000. Actually, the degree of accuracy obtainable depends to a great extent on the weight of sample taken; this is usually 4–6 mgm., but the lower limit of the method for the above degrees of accuracy is 2–3 mgm. On the other hand, if 0·2 gm. of sample or more is used, the error is about 3 parts per 10,000. Much specialised work has been devoted to the investigation of the errors inherent in this method; reference may be made in particular to the contributions of Boetius [11] and of Sternberg,[16] and to p. 33.

Special Modifications of Pregl's Method

Under this heading will be discussed certain modifications of Pregl's method which have been introduced in applying it to some special problems.

Method for Tropical Conditions. Investigations by Nath [31] in India showed that low carbon results are often obtained in the summer months. These may be eliminated by increasing the time of combustion by 5 mins., by passing the gases through the tube at 4–5 ml. per min., and by changing the rubber connexion between the combustion and calcium chloride tubes after each analysis, and the other rubber connexions on the absorption tubes after every three analyses. There should be a minimum of delay in capping the absorption train and transferring it to the balance room, which should be maintained at a controlled relative humidity; and it is advisable to calculate the mean of blank tests made immediately before and after the actual analysis, so as to take into account errors due to any intervening changes in atmospheric conditions.

Metallic Organic Compounds. Determinations of metals are nearly always most accurately carried out on the simple metallic salts (*e.g.*, carbonates). When, however, the metals are present as complex organic compounds or mixed with organic matter, the residue from the

combustion may sometimes be used to obtain a check by the method described on p. 118. Thus, silver, gold and platinum may be determined as metals, even in presence of chlorine, by simply weighing the residue, but the combustion must be carefully controlled, especially at the beginning. Organic salts of iron, chromium, aluminium, copper and tin give good results if the metals are weighed as oxides (Fe_2O_3, Cr_2O_3, Al_2O_3, CuO, and SnO, respectively) after heating strongly in the current of oxygen. Magnesium and lead similarly, can be weighed as MgO and PbO, respectively, but they must not be heated too strongly (see also Holt [44]).

Where the residue cannot be weighed directly several special cases arise :—

(*a*) Compounds containing alkali and alkaline earth metals are best weighed out in an old platinum boat and covered with five to eight times their weight of the purest potassium dichromate, which has previously been just melted, finely powdered, and stored in a desiccator over phosphorus pentoxide. During combustion the tube is protected from contamination due to the spirting of the dichromate by pushing the boat into a cylinder of platinum foil (p. 53), and both containers are extracted with hot water immediately after the analysis. If potassium dichromate is not used, the residue is weighed and converted into sulphate. From this, the carbonate in the residue is calculated, and the corresponding weight of carbon dioxide is added to that as found from the soda-lime tube. If siliceous compounds present difficulties in direct combustion, it is advisable to heat these also in potassium dichromate.

(*b*) Organic metallic compounds, which on combustion yield volatile oxides, or oxides having several stages of oxidation (*e.g.*, salts of manganese, cobalt or nickel) cannot be determined by weighing the residues. Thus, manganese heated in a current of oxygen forms both MnO_2 and Mn_3O_4, whilst cobalt and nickel give mixtures of higher and lower oxides. Zinc oxide is too volatile, and molybdenum forms its volatile trioxide.

Compounds of arsenic, antimony, and bismuth should not be burnt in new tubes, because their oxides attack the filling. According to Furter,[32] large amounts of mercury also poison the filling and, moreover, result in high hydrogen values, because the mercury distils through the neck into the calcium chloride tube.[33] Small amounts of metallic mercury have no noticeable influence on the carbon and hydrogen values, though older tubes are always preferable for the combustion of mercury salts. After a substance containing mercury has been analysed, gold wire foil is placed in the neck of the combustion tube during the subsequent 10–15 analyses.

Other Methods

Under this heading are described briefly certain methods which differ in some fundamental respect from that of Pregl. A number of

such methods have been suggested from time to time, but only those which have some definite justification on grounds of speed, simplicity or accuracy are described. The point made on p. 33 may again be emphasised, namely, that in assessing the relative merits of the Pregl and other methods, the considerable amount of work devoted to the perfection of the former is a great argument in its favour.

Volumetric Determination of the Hydrogen and Carbon. In 1925 Lindner [34] suggested that the carbon dioxide and water produced in the Pregl combustion should be titrated, so as to avoid the tedium and inconvenience of weighing. The water was determined to within $\pm$ 0·05% by passing the combustion products through a mercury valve into α-naphthyl oxychlorophosphine, when the following reaction occurs :

$$R.POCl_2 + H_2O = R.PO_2 + 2HCl.$$

Thus, each molecule of water produces 2 molecules of hydrochloric acid for titration. The reaction is, however, rather slow, and should take place at about 105° C. ; cinnamoyl chloride is preferable in that it reacts more rapidly.[35]

In Lindner's method the carbon dioxide is absorbed in barium hydroxide solution, a procedure which has been developed by Schmitt and Niederl [36] so as to obtain a maximum error of about $\pm$ 0·3%, which is tolerable for many purposes. The only modification to the main Pregl apparatus is the insertion of a platinum gauze just before the tube filling, and of a combined absorption and titration vessel for the carbon dioxide. The latter has the shape of centrifuge tube, and the gases enter it through a narrow tube which reaches almost to the base and is surrounded snugly by an 11-coil spiral tube. The tips of two Pregl burettes holding 0·05 *N* hydrochloric acid containing 3% of barium chloride, and 0·1 *N* barium hydroxide solution, respectively, are inserted through holes in the stopper of the vessel, which contains initially 0·1 ml. of neutral ethyl alcohol, and 2 drops of a 2% solution of phenolphthalein ; 8 ml. of the barium hydroxide solution are added after insertion of the sample and filling the apparatus with oxygen.

Combustion then proceeds (gas-speed, 3 ml. per min.), followed after 20 mins. by sweeping-out (gas-speed, 6 ml. per min.) with 100 ml. of gas. The titration is then made, while the oxygen is still passing into the vessel. Incidentally, Niederl and Meadow [37] have endeavoured to adapt the barium hydroxide method to work of high accuracy by weighing the precipitated barium carbonate instead of titrating it. Since the weight of the precipitate is more than 16 times that of the carbon it contains, the actual weighing errors are correspondingly small. The limiting factors so far as accuracy is concerned in this case are, however, the conditions of absorption and precipitation.

Friedrich-Dennstedt Method.[38] In Dennstedt's well-known macro-method combustion was carried out catalytically in a stream of oxygen, in presence of platinum contacts in a tube of resistance glass ; interfering gases were retained by lead peroxide and red lead, and two supplies

of oxygen were used, to make sure of an excess over the platinum. Friedrich has adapted this method very successfully on the micro-scale. Thus he found that when analysing a 3- to 4-mgm. sample an oxygen flow of 4 ml. per min. was adequate if combustion was carried out carefully ; he used lead peroxide at 180°–200° C. to absorb interfering gases. This reagent is contained in two boats, which means that the tube filling is eliminated and the renewals may be made rapidly, after a few analyses ; this is a great advantage, since the lead peroxide often deteriorates before the other contents of the tube.

Other features of this method are the replacement of the usual pressure regulators by a Riesenfeld adjustable gas-speed meter,[39] and the use of a pre-heater for purifying the oxygen, and of sealable absorption tubes without capillaries. This last innovation means that although the choking plug must be retained, the Mariotte bottle is unnecessary, and the analysis can be carried out more rapidly and with a smaller blank than with the usual Pregl method. Belcher [21] reports very favourably on the method, although he prefers to retain the Pregl absorption tubes and Mariotte flask, and uses a White-Wright flowmeter. Incidentally, flowmeters are generally preferred nowadays.

Wet Oxidation Methods for Carbon. Such methods suffer from the drawbacks that hydrogen cannot be determined, and that a reliable universal oxidising mixture has not yet been found ; on the other hand, the technique and apparatus are simpler, and the method is of special value for use with physiological material. Dieterle [40] used a mixture of sulphuric acid and potassium dichromate. The products of decomposition were carried through an ignited layer of potassium chromate and copper oxide in the stream of oxygen ; the carbon is thus oxidised completely to carbon dioxide, which is determined gravimetrically. Houghton [45], using phosphoric acid in addition to Dieterle's reagents, and the volumetric procedure for determining the carbon dioxide, has evolved a simple and efficient method which gives good results with many substances. However, it is not universal in its application ; halogens, in particular, interfere and they must be first removed. Lieb and Krainick [41] added Lindner's silver dichromate catalyst to the oxidising mixture, and led the products of combustion over red-hot platinum contacts into barium hydroxide solution (see Lindner's method, p. 61). Schadendorff and Zacherl [42] adapted this method to biological materials. The nearest approach to the ideal universal oxidising mixture is, however, the Van Slyke-Folch reagent recommended by McCready and Hassid,[43] namely, chromium trioxide, potassium iodate, phosphoric acid and fuming sulphuric acid ; it enables a determination to be made in 30 mins.

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DETERMINATION OF NITROGEN

The Micro-Dumas Method

This, again, is a micro-adaptation of a classical macro-method, the principle of which is the production of gaseous nitrogen when a nitrogenous compound is heated with copper oxide in presence of a stream of carbon dioxide and reduced copper metal ; the gas evolved is measured directly in a nitrometer. Here again, too, the pioneer work on the micro-method is due to Pregl, and most of the later methods of this type have been modifications of the original, designed to improve accuracy and rapidity of operation. The principal points to be watched are that the supply of carbon dioxide is pure, and, especially, free from atmospheric nitrogen ; and the errors associated with the copper spiral and the copper oxide (see below). The method is usually accepted as reliable for the determination of most forms of nitrogen. The few exceptions (see p. 77) may be determined by the micro-Kjeldahl method (p. 78), which is considerably quicker but which also will not give reliable results with all types of nitrogenous

compounds. The Pregl micro-Dumas method has, however, been so perfected and simplified that it is no longer the province of the expert; it is even sometimes taught to students in preference to the macro-Dumas method.

Historical

In Pregl's first micro-combustion tubes the reduced copper, in spiral form, was placed at the end of the combustion tube as in the macro-Dumas method. This method took about 20 mins. and gave accurate nitrogen values in expert hands, if 10% was deducted from the volume of the gas collected. Of this excess about 2% was due to the space occupied by the 50% potassium hydroxide solution adhering to the walls of the nitrometer; the remaining 8% arose from causes such as the formation of carbon monoxide and, possibly, also of other associated gases.

The reduced copper spiral is also a possible cause of the increase in volume of the gas. Thus, when it is heated in a tube through which carbon dioxide is passed slowly, an increase in the size of the bubbles rising in the nitrometer is apparent, owing to a disturbance of the equilibrium between carbon dioxide on the one hand and carbon monoxide and oxygen on the other. Pregl, therefore, placed the reduced copper in the middle of the tube, filled up the latter to the neck with a larger amount of copper oxide, and allowed the combustion tube to project for some centimetres beyond the tube-burner; a fall in temperature thus occurs in the projecting portion of the tube, and facilitates the complete oxidation of any carbon monoxide, so that no measurable volume of gas comes from the heated tube during an experiment lasting 30 mins. Thus, 0·2-mm. bubbles at the rate of 1 per sec. total a volume of only 0·014 ml. in 1 hr.

It has also been noted, that if a tube filled with copper oxide only is alternately heated and cooled, rather larger bubbles (which again become small on cooling) will be formed in the nitrometer; this appears to be due to dissociation of carbon dioxide. The fall in temperature produced by allowing the tube to project 40 mm. beyond the tube burner completely prevents this.

Finally, during the combustion of organic substances nitrous oxide may also be obtained. This will not itself affect the result, as nitrous oxide occupies the same volume as the nitrogen which it contains. Since, however, at very high temperatures it dissociates into nitrogen and oxygen

$$2N_2O = 2N_2 + O_2,$$

unless the latter is completely absorbed some of it will collect in the nitrometer. This is prevented by using relatively large quantities of reduced copper in the hottest part of the combustion tube, and by not exceeding the prescribed bubble-rate of one in 1·5 sec.

Flaschenträger [1] has observed that coarse copper oxide is occasionally the cause of high nitrogen values, owing to occlusion of air. He recommends leaving the coarse copper oxide of the moveable filling in

the tube for several analyses. Ogawa [2] confirmed this observation, and removed the air from the hollow spaces by evacuating the tube and subsequently filling it with carbon dioxide, by the method of Berl and Burkhart.[3] Hernler [4] allows the coarse and fine copper oxide to cool in a Kjeldahl flask after repeated evacuations under pure carbon dioxide. According to Trautz,[5] the permanent filling involves no source of error due to enclosed air, if the tube is always under a slight excess pressure of carbon dioxide. The removable filling, on the other hand (since it consists of the usual copper oxide which has been ignited and cooled in air, and the carbon dioxide taken from the Kipp apparatus), is an almost constant source of gas, because for every analysis about the same amounts of carbon dioxide and copper oxide are used. An approximately constant correction of 0·003–0·006 ml. of nitrogen for each analysis (*i.e.*, about 1% of the volume of nitrogen which is normally obtained) is then applied. A second correction is proportional to the volume of nitrogen collected, and comprises 0·5% for the volume diminution due to the potassium hydroxide solution and 0·3% for its vapour tension (Trautz). Friedrich [6] evacuates the filled tube and then allows the carbon dioxide to stream in. After three such operations only 0·5–1·0% of the total volume need be deducted, *i.e.*, for the volume diminution due to the alkali. Experiments have confirmed these observations, but it is, however, not only a question of the volume diminution due to the alkali solution; and by reason of the 1·0–1·2% of air retained by the copper oxide of the removable filling (which is independent of the volume of nitrogen obtained), the Pregl correction of 2% should be retained. It is, however, exact only when the "constant error" is 1·2% of the gas volume, *i.e.*, when 0·3–0·5 ml. of nitrogen is obtained by Pregl's technique.

For the first determinations with azobenzene, combustion tubes with new permanent fillings gave results too high by about 0·3–0·5%, owing to the adsorbed gases. If, however, the tube is kept permanently filled with carbon dioxide, the values obtained after two or three analyses are excellent. Before the first test analyses, therefore, it is advisable to ignite new tubes strongly three times in a slow stream of carbon dioxide (1 bubble per sec.); ten tubes so pre-treated gave nitrogen values for the first test analyses on azobenzene which differed from the theoretical by less than $\pm$ 0·1%.

Apparatus

Attention will be devoted in the first instance to the classical Pregl apparatus, and some of its minor modifications. This has been the subject of detailed dimension specifications drawn up by a committee of the American Chemical Society.[7]

Carbon Dioxide Supply. As already mentioned, this is a highly important feature of the apparatus, because the gas used must be very pure. For this reason Pregl's Kipp generator (*vide infra*) which requires careful preparation, is now being replaced by solid carbon dioxide

(" dry ice ") in a Dewar flask ; thus a 1-litre flask will hold about 2 weeks' supply (3 lb.). Care must be taken that all the air is first removed from the container, and it usually suffices to allow evaporation to proceed for 24 hrs. before using the gas. Carbon dioxide from cylinders may also be used,[8] but care must be taken to purify such gas. In any case it is important to ensure the specified requisite pressure in the apparatus, as variations may affect the result.

Pregl's Kipp generator and the method of preparing it will now be dealt with in detail. The gas is liberated from pure marble and acid, but in order to obtain the requisite micro-bubbles of gas, commercial marble is broken up to the size of hazel nuts, etched with dilute hydrochloric acid, washed, covered with water, and boiled for 10 mins. After cooling, it is placed in a large desiccator and covered with calcium chloride solution, which has been syphoned off from an exhausted Kipp generator and completely neutralised by adding more marble. The desiccator is evacuated, by means of the water-pump, to remove the air from the pores of the marble. If, after 30–60 mins. no more bubbles escape from the marble, the vacuum is slowly released so that the pores fill with calcium chloride solution. In order to remove the last traces of air, it is again evacuated for 30 mins., after which the marble is ready for use.

All the air is now removed from the micro-Kipp apparatus (Fig. 42), as quickly as possible, through the vertical tube attached to the stopcock, which draws from the highest point of the middle bulb (see *h*, Fig. 42). This bulb is now half-filled with the treated marble ; short glass rods, or a perforated glass cylinder encircling the centre tube, are better than leather or rubber discs for preventing the marble from dropping into the lower bulb. The rubber stopper carrying the stopcock is now moistened with glycerin and inserted. Beyond the stopcock it is advisable to fuse a bulb filled with cotton-wool, which connects with a glass tube of the same bore ; this keeps back the mist of hydrochloric acid carried over by the carbon dioxide. The thick-walled capillary R (thermometer tubing) is connected to the delivery tube of the Kipp apparatus by means of a very tight piece of pressure tubing, about 100 mm. long, which is moistened with glycerin. The two glass tubes are in contact inside the rubber, and they are of the same diameter ; the rubber tubing is wrapped round with several strips of paper to prevent cracking. The other end of R forms a tapered capillary, and fits into the mouth of the combustion tube through a rubber stopper. One litre of pure, fuming hydrochloric acid, diluted with the same volume of tap water, is used to fill the lower bulb and about one-third of the upper bulb.

If the cock H_1 (Fig. 42) is now opened, air is expelled from the middle bulb and carbon dioxide is liberated by the acid which enters it. However, the acid still contains a large amount of air in solution, and two to three lumps of marble of the size of filberts are therefore dropped through the upper bulb, into the central tube, and there develop

carbon dioxide in abundance. The air is thus completely removed from the hydrochloric acid in the upper bulb, fresh acid being admitted to the upper bulb by repeatedly opening and closing the cock. A

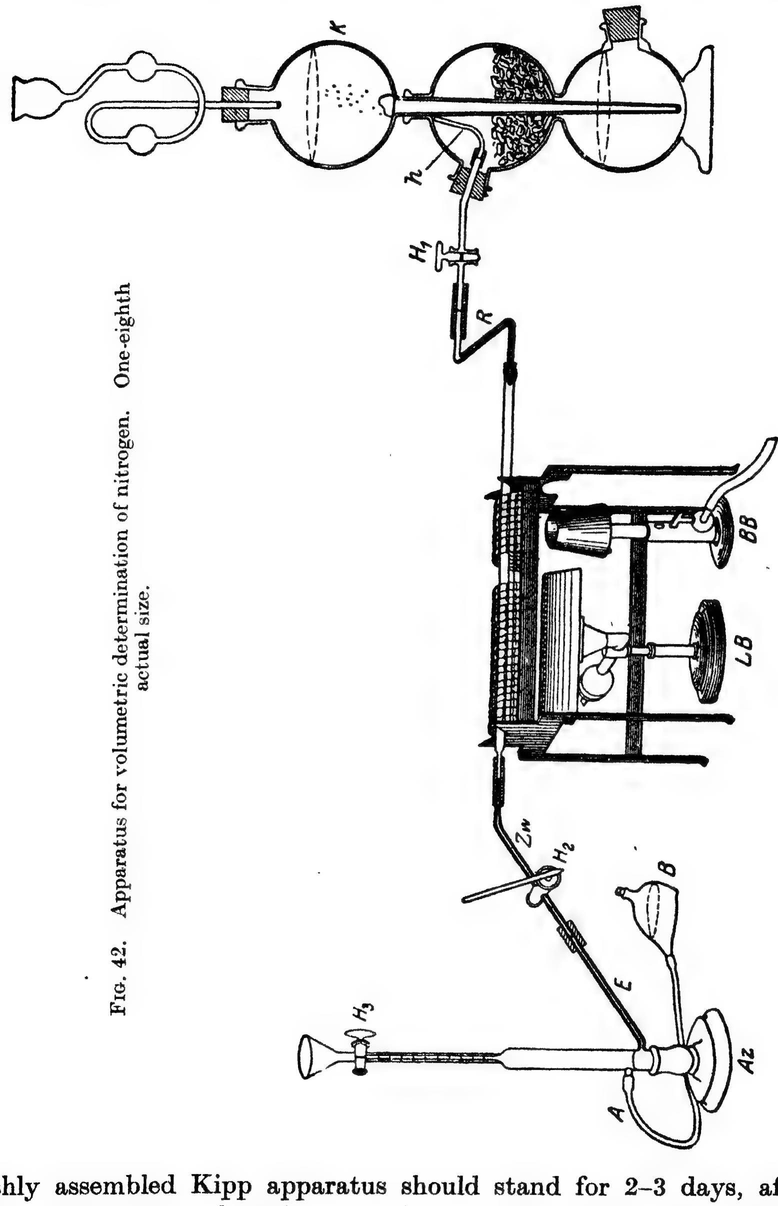

FIG. 42. Apparatus for volumetric determination of nitrogen. One-eighth actual size.

freshly assembled Kipp apparatus should stand for 2–3 days, after which fresh lumps of marble are added to the upper bulb. This is because new generators contain appreciable amounts of air, not only on the surfaces of the glass, but also in the rubber stoppers, and this is liberated only after exposure for some days to the carbon dioxide. For apparatus which has already been used and is immediately refilled

after being completely cleaned, one day of exposure suffices. Generators should also be deaerated after standing overnight.

Hein's apparatus [9] (Fig. 43) avoids the constant contact of the acid with the atmosphere. Its upper bulb is closed to the air of the room by a mercury valve, and the gas-filled portion of the middle bulb is connected with the upper bulb through a tube which includes a glass cock. This arrangement makes air-free carbon dioxide available as required much more rapidly. Its manipulation, however, requires care and experience.

Conditions are satisfactory when the carbon dioxide is almost completely absorbed by the potassium hydroxide solution, so that the bubbles vanish leaving a scarcely perceptible trace. If the micro-bubbles are led for 30 mins. into the nitrometer, at a rate of 1 bubble per sec., few bubbles should be visible below the tap H_3 (see p. 71).

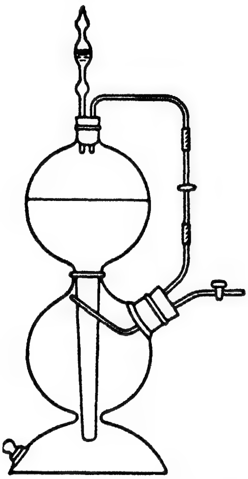

FIG. 43. Kipp generator, with mercury valve.

When the acid has become weakened by use, it is advisable to empty the generator so that no air enters the middle bulb, *i.e.*, by syphoning the acid from the upper bulb after the acid has risen into it, or by running off the acid through a glass cock which is inserted in the stopper of the lower bulb. Half of the acid drawn off is replaced by the same volume of pure concentrated hydrochloric acid, and the used acid serves for refilling the generator. The calcium chloride present reduces considerably the coefficient of absorption of the solution for air.

The Combustion Tube is 500 mm. long, and is provided with a neck. An ignited wad of asbestos is pushed in up to the neck, with a glass rod, and there compressed to 5–6 mm. Copper oxide wire is then introduced (130 mm.), and this permanent filling is held in position by means of a gently compressed asbestos plug. A 40-mm. roll of wire is now passed over the tube, which is placed on a combustion stand, as in the determination of carbon and hydrogen (p. 36). A 40-mm. layer of copper oxide is then reduced to metallic copper by heating in a current of hydrogen, which has been passed through potassium permanganate solution ; begin at the asbestos plug in the middle and move the burner slowly up to the neck.

It is advisable, before using the tube with its permanent filling for the first time, to pre-heat it three times in a slow stream of carbon dioxide and to allow it to cool in this gas (*cf.* p. 74). When not in use, the tube is left on the combustion stand, still connected with the Kipp generator, so that it contains carbon dioxide under pressure. The copper oxide need not then be freshly reduced or renewed until after several hundred analyses. By always keeping 10 tubes filled with carbon dioxide under pressure, the further advantage is gained that air

is more quickly removed before analysis, and for both reasons the use of the Kipp generator is favoured rather than heating sodium bicarbonate; the latter method has the further disadvantage that volatile substances may undergo considerable loss in a current of warm carbon dioxide.

The permanent filling is followed by a removable filling of fine copper oxide, which is renewed for each analysis; or by coarse copper oxide (before the reduced copper), which projects 3–4 mm. over the tube burner and which must be renewed at once if it shows even partial reduction. This filling protects the permanent filling, to a certain degree, from the occlusion of air after ignition; therefore the time of passing carbon dioxide before combustion is shortened.

To fill the combustion tube remove the prepared rubber tubing (see p. 39) from the neck, disconnect at *R*, and empty the removable filling into a stock bottle. Fresh copper oxide wire (p. 72) is then introduced (length, 90–100 mm.), by scooping it out from the wide-mouthed bottle with the combustion tube; this is omitted if the permanent filling has been placed adjacent to the fine copper oxide (see p. 72). Finely powdered copper oxide is poured in on top of this filling (length, 5–10 mm.) to prevent any particles of the sample being analysed from falling into the coarse filling, and thus undergoing premature combustion. The sample is intimately mixed with copper oxide in a mixing tube (p. 73) by vigorous shaking, and then transferred to the tube through a filling funnel (Fig. 44), which is prepared by drawing out a test-tube in the middle to a diameter of 5 mm. and a length of 60 mm. To remove the remaining sample from the mixing tube more copper oxide is scooped out of the stock bottle with the open end of the tube, which is again corked, well shaken, and emptied into the combustion tube. This operation is repeated three times more, and there should then be in the combustion tube a 90-mm. layer of fine copper oxide. A final layer (10–20 mm.) of coarse copper oxide is added.

FIG. 44. Filling tube. Actual size.

It is sometimes found that the development of static electricity on shaking makes the quantitative transference of the sample impossible. Many workers, therefore, now prefer to weigh the sample out in a porcelain or platinum boat (p. 18) which is transferred directly to the tube, 50 mm. of fine copper oxide being inserted similarly on the coarse copper oxide. The boat is allowed to slide along the tube on to this layer, and is covered with a 40- to 50-mm. layer of the fine oxide, and finally with a 10- to 20-mm. layer of coarse copper oxide. Capillary tubes containing liquid samples are pushed into a roll of freshly oxidised copper wire gauze (length, 40 mm.; internal diameter, 5 mm.) immediately after breaking off the handle and tip. The whole is allowed to slide into the tube, and a layer of coarse copper oxide is quickly poured on it as usual.

After covering the combustion tube with two closely fitting rolls of wire gauze (150 mm. long for the tube burner, and 40 mm. long for the moveable burner) the tube is placed on the combustion stand so that the neck still projects 40 mm., and the open end of the tube is connected with the delivery tube from the Kipp generator by means of a perforated rubber stopper (Fig. 42).

The Heating System calls for no special comments beyond the points mentioned in connexion with the determination of carbon and hydrogen (p. 36). Attention should, however, be drawn to the automatic method of Royer, Norton and Foster,[8] which has the double advantage of ensuring reproducibility of procedure (and therefore of results) and of requiring the minimum of attention ; thus whilst an analysis takes 40 mins., the worker's attention is required for only 20 mins.

Both the stationary and moveable heating systems are electrically heated, and are supported on carriages sliding on a horizontal bar. The former is locked in position, and the latter is moved progressively during the analysis by means of a two-speed, governor-controlled gramophone motor. The actual furnaces are made from platinum resistance wire coated with alundum cement, and have refractory tube-shaped linings which are in two portions so as to enclose the tube completely when the lids of the furnaces are in position. The ends of the furnaces are so designed that the refractory portions join when the moveable portion reaches the fixed portion at the end of its travel.

The Precision Micro-Nitrometer (*Az*, Fig. 42) is a micro-version of the usual type. The scale has its zero-point at the cock H_3 ; it begins at 0·05 ml. and usually covers 1·2 or 1·5 ml. The spaces between the divisions correspond with 0·01 ml., and the volume can easily be estimated to 0·001 ml., particularly with the help of a reading lens. The separate scale-divisions extend round three-quarters of the circumference of the tube, making it possible to read the volume without error due to parallax.

The Haack type of micro-nitrometer is inverted, and is calibrated with mercury from the stopcock to the scale-divisions concerned. The resulting convex mercury meniscus and the concave meniscus obtained with potassium hydroxide solution almost coincide. The Physikalische Technische Reichanstalt, Charlottenburg, has found that the volume with mercury is the smaller by 0·001 ml. Such nitrometers are etched with the mark " KOH." Nitrometers marked " Hg " should not be used. They are calibrated with mercury in the upright position, and show a difference of about 0·007 ml. at each scale division.

Care should be taken that the gas inlet tube should retain its bore of 0·8–1·0 mm. at the point where it is sealed on, and not be widened by sealing as this produces large bubbles. Since the velocity of the combustion is regulated according to the bubble frequency, a considerable error may thus arise. Most precision micro-nitrometers are supplied with a certificate of standardisation.

A suitable wooden stand, and a metal fork attached by a screw below the funnel (to support the levelling vessel at a high level), are also necessary. A further important accessory is the connexion *Zw*, with a cock H_2 which has a long handle to provide a sensitive adjustment. The best design for H_2 is that recommended by the American Specification Committee [7]; the direction of the groove on the lower end of the straight bore is away from the opening of the bent bore, so that a safety zone of at least one-fourth of the circumference of the stopcock plug is available. The bores are 1·50–2·0 mm., widening to 2·0–2·5 mm. at the air outlet, and the handle is 63–67 mm. long.

If water collects in *Zw* (*e.g.*, during the combustion of compounds rich in hydrogen) then it, with the stopcock, is rinsed with alcohol and dried with the pump to prevent obstruction. If *Zw* is provided with a ground-in joint in which cotton-wool is placed, this prevents water and copper oxide dust from passing over into the barrel of the cock, and no readjustment of the bubble velocity is necessary. Before filling, the nitrometer is cleaned by rinsing it repeatedly with chromic acid in sulphuric acid and water, and is allowed to drain in the inverted position. The levelling vessel *B*, which has also been cleaned and dried, is then connected with the side-tube by means of rubber tubing both ends of which are bound with wire. Mercury is added through the levelling vessel until the level is midway between the inlet tube *E* and the upper side-tube *A*. The cock H_3 is then carefully lubricated with a little vaselin. Other lubricants cause the 50% potassium hydroxide solution to foam after a short time. Sufficient of the latter is added to fill the measuring tube and about one-third of the pressure tube.

Sometimes, when nitrometers which have been filled with pure mercury and pure 50% potassium hydroxide solution are first used, the rising gas-bubbles are held at the boundary between the mercury and the solution, and rise only after considerable shaking. This difficulty, however, ceases after the first determinations, when sufficient finely divided copper oxide dust has collected at the boundary between the two liquids. Nickols [10] stimulates the rise of the bubbles by the addition of mercuric oxide; Trautz [5] adds a few drops of mercury which have been extracted successively with ether, water and potassium hydroxide solution.

As Trautz has shown,[5] the normal errors due to the nitrometer are small and relatively constant; certain improvements resulting also in improved strength, less cost, and ease of repair are, however, intoduced into the mercury nitrometer proposed by Clarke and Winans.[11] The principle on which it is based is measurement, by weighing the nitrometer, of the amount of mercury displaced by the nitrogen evolved; in this way it is possible to arrange that the alkaline solution does not come into contact with a stopcock, and since 0·001 ml. of nitrogen is equivalent to 0·013 gm. of mercury, an accuracy in

weighing of $\pm$ 0·01 gm. suffices. The apparatus consists of three units connected in series, viz., an absorption tower, the mercury nitrometer proper and a reservoir to receive the displaced mercury and to enable the liquid levels to be adjusted. During the actual combustion only the absorption vessel (the outlet from which is closed) is connected to the combustion train. After combustion is complete the nitrometer (which has been weighed) and the levelling vessel are connected to the system by ground glass joints, and the level of the alkaline solution is restored to the position it occupied originally. This involves displacing some mercury from the nitrometer into the levelling bulb, so that by disconnecting the former and weighing it the mercury lost, and thence the nitrogen evolved, may be found.

Gull [12] also describes a gravimetric method in which the nitrogen is collected in the nitrometer in the usual way, and then expelled into a small weighed flask containing water, and fitted with a side-arm ; the flask is inverted so that the side-arm is over a jet in the funnel at the top of the nitrometer, and the weight of water displaced by the gas is found by reweighing the flask. The method is accurate once the technique has been acquired, but it is not recommended for the beginner.

Reagents

Copper Oxide and Metallic Copper. Two forms of *copper oxide* are used for filling the combustion tube (see p. 69), and are kept in wide-mouthed bottles with ground-in stoppers. (1) Wire-form copper oxide, which is crushed in a mortar before use in order to obtain pieces 4–5 mm. long ; (2) finely divided copper oxide, which is obtained by grinding (1) in a mortar and sieving it. Very finely powdered or precipitated copper oxide cannot be used, as they offer too much resistance to the gas-flow.

The *metallic copper* may be prepared by reduction with hydrogen when filling the tube. Where large quantities are required it is advisable to reduce a large amount of the wire-form copper oxide completely, with hydrogen in a combustion tube, and to maintain a stock of this material for tube-fillings. The filling is removed from the tube after every analysis into a bottle having a wide mouth, and accumulations from about five fillings are ignited in a nickel dish for 15 mins. After cooling, the coarser and finer portions are separated by means of a fine-meshed sieve, and stored separately for further analyses.

Potassium Hydroxide Solution. It is absolutely necessary in order to be able to read the small gas-volumes, that the level of the 50% potassium hydroxide solution in the nitrometer should be completely free from foam. Two hundred grams of potassium hydroxide in sticks, dissolved in 200 ml. of water, are treated with 5 gm. of finely powdered barium hydroxide. After shaking, the liquid is allowed to stand for 15 mins. to allow most of the precipitated barium carbonate to settle.

It is then filtered through an ordinary dry filter paper, and stored in bottles having rubber stoppers.

Carbon Dioxide. See p. 65.

Procedure

Weighing out the Sample. This procedure has already been dealt with in general terms (p. 16), but some further notes specially applicable to the present determination are desirable.

Solid substances are usually weighed in weighing tubes with long or short handles (Fig. 45); these can be made very easily (Lieb and Krainick [13]). If the weight of the shorter tube is a few tenths of a milligram over 500 mgm., a 500-mgm. weight serves as a counterpoise, so that complete weighings can be undertaken with the rider alone. The counterpoise of the longer tube is just over 1,000 mgm.; this tube is weighed on the hook of the balance. If residues stick to the tube,

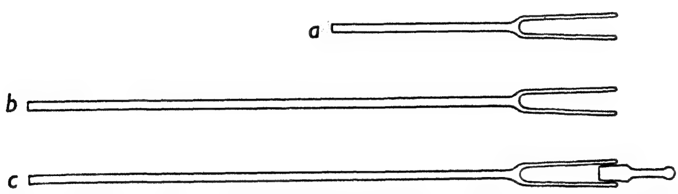

FIG. 45. Weighing tubes for nitrogen determination, with (*a*) short handle, (*b*) long handle, (*c*) ground-in stopper. Actual sizes.

it is cleaned with alcohol and acetone, dried by drawing it once through a non-luminous flame, wiped with chamois leather and placed near the balance. For weighing hygroscopic substances ground-in stoppers (Fig. 45, *c*) are used.

Oily and syrupy substances are weighed, as for the carbon and hydrogen determinations (p. 53), in porcelain or platinum boats, and are covered to the edge of the boat with fine copper oxide. Liquids are weighed by the method described on p. 17, a crystal of potassium chlorate being fused in the capillary and the capillary closed. With low-boiling liquids the use of unnecessarily large capillaries is to be avoided, because the nitrogen of any air occluded is obtained in the nitrometer after the combustion.[14]

As a rule 2–5 mgm. of material are weighed out; this should yield 0·3–0·5 ml. of nitrogen. With substances very rich in nitrogen about 1·5 mgm. are taken, and the weighing is carried out with the greatest possible accuracy. Substances containing less than 2% of nitrogen, or which are being examined for traces of nitrogen, are exceptional; in order that the limiting error of 0·2% is not exceeded, 8–10 mgm., but not more, should be used.

After the weight of the tube filled with the material has been noted, the latter is emptied into a mixing tube made from a micro-bomb tube (p. 98), which is cut down to a length of 70–80 mm.; the sharp edges are carefully rounded with fine glass-paper and smoothed in the

flame. On transferring the material, the weighing tube is held obliquely by the handle in a chamois leather in the left hand, and taken from the balance with the mouth held uppermost; the mixing tube is held horizontally in the right hand. The mouth of the weighing tube is pushed 1–2 mm. into the mixing tube, and inclined until the material falls into the latter. If the substance sticks fast, the weighing tube is gently tapped or rotated. The two tubes are then again held horizontally, one inside the other, and the last traces of the sample gently tapped into the mixing tube. The sample is covered with 20 mm. of fine copper oxide, and the mixing tube is closed with a non-porous cork. After 5 mins. the empty weighing tube is weighed again to ascertain the amount of substance used.

If the sample is very hygroscopic, it is dried in a capped weighing tube, an appropriate quantity then being transferred rapidly to a porcelain boat in which it is mixed with 40- to 80-mesh copper oxide, with the boat in the combustion tube itself. The weighing tube is reweighed to find the amount removed.[15]

The procedure from this stage is described under the preparation of the combustion tube (p. 69).

The Combustion. The stop-cock H_1 (Fig. 42) is fully opened, and carbon dioxide is passed through the tube for 3 mins. before inserting the nitrometer, in order to displace the air from the filling. Then the nitrometer is connected, as shown, with the cocks H_2 and H_3 open. After closing H_2 the nitrometer is filled with potassium hydroxide solution by raising the levelling vessel to the height of the cock H_3, so as to replace the air in H_3 by solution; H_3 is closed, the levelling vessel lowered, and by slowly opening the cock H_2 a bubble-frequency of 3–4 bubbles per sec. is set up. The combustion tube is now heated to redness.

Observation with a lens is used to decide whether sufficiently small micro-bubbles are being obtained; they have a very much slower rate of rise in the nitrometer, as compared with that of the larger bubbles, and they rise slowly, and at short intervals from one another, in line. Nothing is gained by passing carbon dioxide for a longer time, and it is actually inadvisable to do so with substances which are appreciably volatile, as the vapours of the material will be prematurely burned and the nitrogen values will be low; this was noted repeatedly with nitrosodimethylaniline and dinitrotrichlorbenzene. Consistently correct values were obtained regularly with these materials when the current of carbon dioxide lasted long enough to produce bubbles of the correct size.

The combustion is now started by first closing cock H_1 of the connecting tube, and fully opening cock H_2. The small roll of wire gauze is brought over the end of the copper oxide layer, and the moveable burner is placed below it so that the short roll is in the hottest zone of the flame, which is now fully on and just non-luminous. The heating of the tube at once gives rise to renewed evolution of gas

bubbles into the nitrometer; this slackens after some time if the position of the burner is not changed. The levelling vessel is now raised somewhat higher than the cock H_3, any foam is taken off in the funnel, and the levelling vessel is placed at the base of the nitrometer, where it remains throughout the combustion. The small roll and the moveable burner *B* are now moved slightly towards the material to be analysed, and left at this point until the evolution of bubbles begins to slacken. The gauze roll and burner are then moved forward again, taking care that not more than 2 bubbles in 3 secs. enter the nitrometer; namely, by moving the burner only a few millimetres when in the neighbourhood of the sample, and moving it forward only when the frequency of the bubbles is less than the above maximum. When the whole section filled with the sample has been heated as described and the bubble-frequency begins to fall on bringing the moveable burner further forward, this burner can be moved forward more rapidly until it is immediately behind the tube burner *LB*.

The cock H_2 is then closed, the cock H_1 of the Kipp generator is opened, and the cock H_2 is adjusted very carefully with the help of the long handle so that 2 bubbles or less rise in 3 secs. This operation requires some practice, because the specified gas flow must not be exceeded for even a few seconds, or high nitrogen values will result. The period during which the gas is expelled is utilised by once more igniting the whole length of the tube with the moveable burner for 10 mins. It suffices to place the burner at about four different points along the section to be ignited, from the end of the filling up to the tube burner. The expulsion period can be shortened by extinguishing both burners as soon as the gas bubbles begin to become smaller, and increasing the gas velocity to 1 bubble per sec.

After some time it will be noticed that the gas bubbles in the nitrometer gradually become almost as small as those described above as micro-bubbles. The determination may now be stopped, for it has been shown by calculation (p. 64) that the volume of the micro-bubbles is too small to affect the result. From the beginning of the combustion to this stage takes 30–40 mins., according to the quantity of nitrogen in the material. The levelling vessel is now supported in the metal fork near the funnel, and the nitrometer is removed, the cock H_2 being closed before removing the rubber tubing of the tube *Zw* from the combustion tube. The warm combustion tube is closed at once with a rubber cap, so that it cools in carbon dioxide under pressure from the generator.

The nitrometer is placed in a sheltered position (in summer it must sometimes be brought into a cooler room, so that the gas reduction Tables may be used), and a thermometer is hung over the funnel just touching it but immersed in the solution. If bubbles or froth have accumulated at the meniscus, the rubber tubing leading to the levelling vessel is pinched with the thumb and forefinger, and that leading to the nitrometer is sharply tapped with the other hand, so

that the potassium hydroxide solution is forced into the nitrogen and the froth bubbles are broken. Then the cock H_2 is opened for the purpose of reading the volume of the gas under the prevailing atmospheric pressure.

Reading the Nitrogen Volume. Temperature equilibrium is established 15 mins. after disconnecting the nitrometer, and readings are taken of the temperature to $\pm$ 0·5° C. ; of the barometer, to $\pm$ 1 mm. ; and of the gas volume in the nitrometer to $\pm$ 0·001 ml. For this purpose the lens is slid up to the level of the meniscus, the nitrometer is held by the funnel with the right hand, while the levelling vessel in the left hand is raised until the liquid in it is at the same height as the meniscus. Parallax should be avoided by so adjusting the lens that the scale-divisions adjacent to the meniscus coincide with their continuation round the back of the tube. It is necessary to avoid holding the measuring tube or placing it near a lighting unit during the reading, owing to the rapid expansion of the gas.

Occasionally, after long use, the potassium hydroxide solution leaks through the closed cock H_3, owing to deterioration of the lubrication ; the reading then gives too high a result. The liquid which has penetrated can, however, be forced back into the funnel without the slightest loss of gas by raising the levelling vessel above the level of the solution in the funnel and carefully opening the cock H_3 ; or by pressing the cock in firmly and tapping down the solution by short strokes on the rubber tubing, as previously described.

Calculation

For reasons explained on p. 64, 2% of the volume (v_a) of gas read must be subtracted from it ; the difference is the true nitrogen volume v. The logarithm of the weight (x) of 1 ml. of nitrogen at the recorded temperature t° C., and barometric pressure p mm., is obtained from gas reduction Tables (*e.g.*, Küster's logarithmic tables, see p. 146). The percentage of nitrogen in a weight, s gm. of the sample is :

$$\log (\%\mathrm{N}) = \log v + \log x + 2 - \log s.$$

Example :

$$2{\cdot}280 \text{ mgm. nitrobenzene} : p = 712 \text{ mm.}, t = 22° \text{ C.}$$

$$v_a = 0{\cdot}246 \text{ ml.} ; v = 0{\cdot}241 \text{ ml.}$$

$$C_6H_5NO_2 \begin{cases} \text{Theory, } 11{\cdot}38\% \text{ N.} \\ \text{Found, } 11{\cdot}46\% \text{ N.} \end{cases}$$

Notes and Errors

The determination of nitrogen as described requires minor modifications in exceptional cases. Firstly, attention should again be drawn to the removal air before combustion, in the shortest time possible in the case of volatile liquids or solids of high vapour pressures. Low nitrogen values are obtained unless particular care is given to the bubbles rising in the nitrometer during heating with the moveable burner ; or if the froth in the funnel is removed too late. Infusible

substances, combustible with difficulty, must be finely powdered in an agate mortar and mixed with an excess of fine copper oxide. The admixture of substances which form almost incombustible nitrogenous charcoal with copper acetate, or with about three times their weight of potassium chlorate and/or potassium dichromate (as in macro-analysis) gives no certainty of correct results; this is because the potassium chlorate decomposes before the complete oxidation of the nitrogenous charcoal. A large excess of potassium dichromate must be used to ensure complete oxidation.

With the usual roll of wire gauze, the copper oxide filling in the upper part of the tube opposite the flame is not sufficiently heated. The roll may therefore be cut through in the middle, and a roll of platinum foil of the same length attached to one half. The upper half of the roll so formed is covered with asbestos paper, in order to minimise heat loss. During the determination, the roll of wire gauze is fixed over the tube and serves as a pre-heater, and the platinum roll is heated in a non-luminous flame.

According to Wiedemann,[16] the combustion should first be carried out normally until the period of expulsion commences. The tube is then allowed to cool for about 5 mins., after shutting off both burners, and the region containing any insufficiently burnt residue is carefully tapped, so that the empty spaces formed during the combustion by the evolution of gas fall together and the unburnt residue again comes into contact with copper oxide. If this is now ignited further there is no residue. This method has proved excellent for the analysis of plant substances, so long as the micro-bubbles are as small as possible.

Errors. Certain of these, which became apparent in the early work on the method, are dealt with on p. 64. Trautz [5] has investigated these further. Thus, the carbon dioxide may dissociate into carbon monoxide and oxygen, which are not absorbed by the alkali unless the conditions of heating and the gas speed are controlled as described, and corrected for. Air dissolved in the acid used in the Kipp generator may also be evolved, and gives rise to error. It should be determined and corrected for, but if it exceeds 4 cu. mm. per 100 ml. of gas, a gas meter must be inserted between the generator and combustion tube so that a correctly measured volume of gas is used, and the correction is correspondingly accurate. Under these conditions it is often possible to speed up the gas-flow by using 4 bubbles per sec., thereby reducing the time required for an analysis to 15–20 mins. The amount of nitrogen absorbed by the alkali is negligible.

The range of the Dumas method is 1–0·03 mgm. of nitrogen. Substances which form tarry or carbonaceous matter on burning (*e.g.*, derivatives of purines, chlorophyll and pyrimidines) often give low results, and their presence is indicated by the continuation of the evolution of bubbles after the normal period [17]; the use of copper acetate at about 1,000° C. often overcomes this difficulty, but the Kjeldahl method (p. 78) or Clark's capsule method [18] are satisfactory;

the latter has enabled nitrogen contents of 0·2% to be determined on 50-mgm. samples with an error of $\pm$ 10%.

The Micro-Kjeldahl Method

Introduction

The Principle of the Method is: (1) Destruction of the substance with sulphuric acid, whereby the nitrogen is converted into ammonium sulphate; (2) steam-distillation of the ammonia liberated by alkali; (3) volumetric determination of the ammonia with 0·01 *N* acid. Very small amounts may be determined iodometrically with 0·005 *N* solutions, or colorimetrically with Nessler's reagent (p. 84).

The first micro-Kjeldahl method was due to Pilch,[19] who decomposed the organic substance with 1 ml. of sulphuric acid, in presence of a crystal of potassium sulphate (to raise the boiling temperature), and 1 drop of mercury as catalyst. Pregl used 1 ml. of sulphuric acid and a mixture of 1 part of potassium sulphate and 3 parts of copper sulphate for the decomposition. With resistant substances the action is accelerated by repeated additions of perhydrol (hydrogen peroxide). Only for albuminous substances did Pregl recommend the addition of mercury. With azo-, nitro- and nitroso-compounds Pregl's method gives low values; if, however, glucose is added to these compounds as a reducing agent, the nitrogen is converted quantitatively into ammonia.[20]

According to Friedrich,[21] hydrazine compounds of the carbohydrates give better values on the addition of glucose, but the results are still not always correct. Hence Friedrich evolved the excellent method of reducing with hydriodic acid, a process which determines hydrazines, dinitrohydrazones, osazones, oximes, nitro-, nitroso-, and azo-compounds, and even certain diazo-compounds, with great accuracy. However, it fails completely with diazo-ketones, R—CO—CHN_2, which split off almost the whole of their elementary nitrogen with hydriodic acid, even the cold.

Much work has been carried out in recent years on the digesting liquor and conditions, and it has enabled these to be adjusted so as to cover a much wider range of substances than hitherto. Mercury is commonly favoured as catalyst, but selenium and copper or a powdered mixture of selenium and mercury [22] or of selenium (3 mgm.) and copper sulphate pentahydrate (4 mgm.) [23] are also effective, so long as the digestion time is not unnecessarily prolonged (*e.g.*, beyond 75 mins.) [24] as this may lead to loss of ammonia. Beatty [25] points out that if the copper sulphate pentahydrate is replaced by the anhydrous salt, several advantages result without loss of accuracy. Thus, owing to its insolubility in the boiling acid bumping is prevented, while the change of the blue colour to black on the addition of excess of alkali is a useful indicator at the distillation stage. The best results are obtained with a mixture of 1 part of the salt, 2 parts of potassium sulphate and 0·2 part of selenium.

Perchloric acid has also been suggested as an aid to wet oxidation because of its rapidity of action ; thus, 20- to 30-mgm. samples require on an average only 25 mins. On the other hand, there is good reason to believe that this reagent gives low results in certain cases, probably by reason of loss of nitrogen as such or by amine formation.[26] Pepkowitz and Shive,[27] however, record very satisfactory results, and there is no doubt that the method offers advantages with the right type of sample and under controlled conditions. They heat 10–15 mgm. of sample with 1 ml. of sulphuric acid and 0·5 ml. of a solution of 12 gm. of selenium oxychloride in concentrated sulphuric acid in a test-tube until the solution is clear (though probably coloured) ; this takes 10–15 mins. Two drops of 35% perchloric acid are then allowed to fall directly into the cooled liquid (to avoid loss of the acid by volatilisation on the sides of the tube), and heat is applied very gently at first, and then more strongly, until the solution is colourless or, if iron is present, tinged light yellow. If it can be established (as Pepkowitz and Shive have done for a number of pure organic compounds and for fertilisers) that the above method gives reliable results for any particular type of sample, it is to be preferred on grounds of speed. If, however, there is any element of doubt, Pregl's method as described below should be adopted.

The advantages of the micro-Kjeldahl method are: (1) Speed and simplicity, especially for multiple determinations ; (2) convenience for the determination of nitrogen in aqueous solutions (blood, urea, etc.) ; (3) accuracy with heterocyclic compounds, which in the micro-Dumas method tend to form nitrogenous charcoals, which are difficult to burn (see p. 77) ; (4) suitability for 0·1–3·0 mgm. of nitrogen.

Apparatus

The Micro-Kjeldahl Flask (Fig. 46) is made of Jena or other resistant glass ; its length is 16 cm., the neck is 15 mm. in diameter, and it is expanded at the lower end to a bulb 30 mm. in diameter.

Digestion Stand. For multiple determinations a stand (Fig. 46) provided with six small burners is used. The decomposition flasks are placed in holes in a heat-resisting plate over the burners, with their necks in a suction device, which is connected with the water-pump. This device is very useful in laboratories which have poor fume cupboards or none at all.

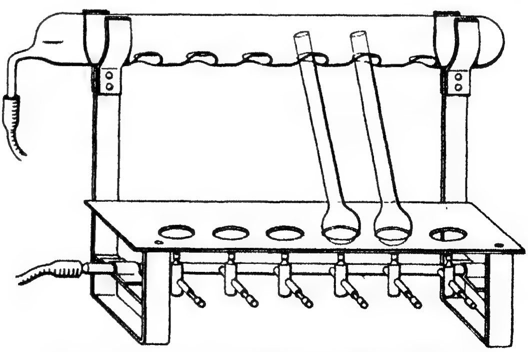

FIG. 46. Micro-Kjeldahl digestion stand.

The Distillation Apparatus suggested by Parnas and Wagner [31] is

shown in Fig. 47. The steam generator, *A*, is a round-bottomed, Jena-type, 1-litre glass flask, which is half-filled with distilled water acidified with a few drops of sulphuric acid. Pieces of pumice prevent delay in boiling, and ensure uniform ebullition.

The trap, *D*, of about 400 ml. capacity, is connected with the steam generator by rubber tubing. In the neck *k* is a rubber stopper through which the steam passes to the distillation flask. At the lower end the trap is constricted, so that any condensed water which collects can be run off at any time through *d*.

The distilling flask, *B*, is of Jena-type glass ; length about 30 cm., external diameter about 3 cm. at the upper half and 5 cm. at the lower.

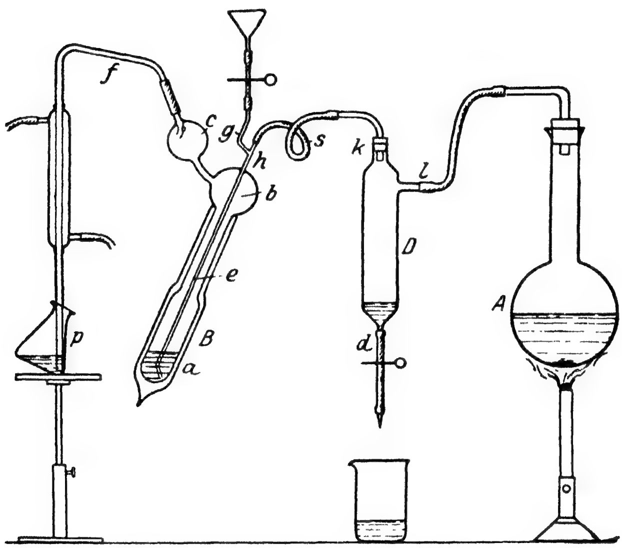

FIG. 47. Micro-Kjeldahl distillation apparatus.

At the top are two bulbs, *b* and *c*, and the sealed-in steam delivery tube, *e*, reaches almost to the bottom of the flask. The flask is entirely surrounded by a glass jacket, *a*, the space between being evacuated. Owing to this thermal insulation the time required for the quantitative removal of the ammonia is reduced to a few minutes. In an earlier apparatus the digestion and distillation were carried out in the same flask, which was fitted to the distillation apparatus after digestion was completed. This had the slight advantage of eliminating the necessity to transfer the solution ; on the other hand, no vacuum jacket could be fitted, and it was necessary to disconnect the flask in order to make the contents alkaline.

The steam inlet tube, which is sealed into the foam-trap, *b*, branches into the arm, *h*, which is connected by the rubber tube, *S*, with the trap, *D*. The other branch, *g*, is connected to a funnel by a rubber tube provided with a pinchcock ; the flask is charged through this funnel. The distilling flask is supported in an inclined position, so that

the delivery tube is approximately vertical; and it is connected by means of rubber tube with the silver condenser tube, *f*, the glass and silver being in close contact. The silver tube is so arranged that liquid condensed in the inclined portion flows back, and not into the distillate. A 100-ml. flask, *p*, which has been pre-treated by prolonged steaming and which contains the standard acid, is placed under the free end of the condenser tube. The use of quartz flasks renders repeated steaming unnecessary, and a quartz condenser tube is as effective as a silver tube, though more fragile and costly.

Reagents

Red Phosphorus.

Hydriodic Acid ($d = 1{\cdot}7$).

Sulphuric Acid ($d = 1{\cdot}84$).

Mercuric Acetate, A.R.

Potassium Sulphate, A.R.

Strong Sodium Hydroxide Solution. This is prepared by dissolving 60 gm. of stick sodium hydroxide in 200 gm. of water, and adding 10 gm. of sodium thiosulphate. The thiosulphate decomposes any mercury-ammonium compounds formed by the decomposition of the sample.

0·01 *N Hydrochloric Acid.*

0·01 *N Sodium Hydroxide Solution.* This is accurately standardised, with methyl red as indicator.

Methyl Red Indicator (*p*-dimethylaminoazobene-*o*-carboxylic acid). A saturated solution in 0·1 *N* sodium hydroxide solution is used.

Procedure

It is very desirable to ascertain in what form the nitrogen is combined, *i.e.*, whether the substance is volatile below the boiling-point of the hydriodic acid or yields volatile products with hydriodic acid, because with certain heterocylic compounds (*e.g.*, antipyrine) and volatile substances the decomposition must be carried out in a micro-bomb (p. 98). With diazo-compounds, which easily split off nitrogen as the element, the Kjeldahl method may occasionally be made possible by coupling with phenol, to form stable azo-compounds; the substance is dissolved in three to four times its weight of phenol on the water-bath, cooled, and treated in the usual way. All other substances are decomposed directly over open flames in Kjeldahl flasks.

Weighing out the Sample. Solid substances which are decomposed in the bomb or in the Kjeldahl flask, are weighed in weighing tubes with long handles (see p. 73). Solids producing marked static electricity are pressed into pastilles (p. 194). Viscous substances are weighed in porcelain boats, which are allowed to slide down the wall of the inclined flask into the bulb. Liquids are weighed in capillary tubes without potassium chlorate. The capillary is slid with the opened tip downwards into the hydriodic acid already in the micro-

bomb, and is there crushed. If the liquid is very volatile, the bomb must be cooled in ice during sealing, and the capillary is not burst.

Physiological fluids (urea, blood, etc.) are transferred to the Kjeldahl flask or micro-bomb with a Pregl 0·1-ml. micro-pipette (p. 31).

Wet-Oxidation (Friedrich's Method). For pre-treatment of the substance in a micro-bomb (p. 98) 1 ml. of the hydriodic acid is allowed to flow down the wall on to the weighed substance, and the bomb is rotated in order to rinse down particles which remain adhering to the walls. Capillaries are then introduced into the bomb. As in the Carius determination (p. 96), the bomb is now sealed and heated in the bomb furnace for 1 hr. at 200° C.; or for compounds, which contain nitrogen atoms adjacent in a ring, at 300° C. After cooling, any hydriodic acid in the tip is driven away by gently warming with a non-luminous flame, the bomb is opened and the contents are rinsed quantitatively with water into the micro-Kjeldahl flask. The procedure is then the same as for substances which have not been pre-treated.

In other cases the substance is weighed directly into the Kjeldahl flask with a few grains of red phosphorus, and 1 ml. of hydriodic acid is added from a measuring pipette; with pre-treated samples only phosphorus is used. The flask is heated (Fig. 47) over a small flame and, after the hydriodic acid has boiled gently for 30 mins., the neck of the flask is washed down thoroughly with water, using sufficient to half-fill the bulb of the flask. Two millilitres of concentrated sulphuric acid are added from a pipette, and the mixture is shaken and heated with a strong flame touching the bottom of the flask, until it boils vigorously; the water and hydriodic acid distil off, and after about 30 mins. the iodine and most of the hydriodic acid have gone, and the solution has become clear. When the neck is free from sublimed iodine, a small amount of mercuric acetate on the tip of a knife and about two to three times its weight of potassium sulphate are placed in the flask. The contents are boiled again for 30 mins., the flame is turned off and the solution is diluted carefully with 2–3 ml. of water.

During the decomposition, the distillation apparatus is steamed out; at first the pinchcocks of the trap and funnel are closed, and a collecting vessel is placed under the condenser. Later the funnel is steamed out by opening the pinchcock, and the silver condenser is rinsed on the outside with distilled water. On removal of the flame a low pressure is set up, which sucks the whole of the liquid in the distilling flask back into the trap. The flame is then replaced and fresh steam generated. Finally, the pinchcock of the trap is opened and the collected liquid is run off.

To 8 ml. (or other suitable volume) of accurately standardised 0·01 N acid in the flask is added a very small amount of the methyl red solution from a glass capillary. The flask is placed under the condenser in an inclined position, so that the end of the condenser tube is well immersed in the acid. The condenser cooling water is then turned on.

Distillation. The solution in the Kjeldahl flask is now transferred quantitatively through the funnel into the distillation flask. It is advisable to grease the outer rim of the neck of the flask very slightly, in order to prevent the solution and rinsing water from creeping over ; and to rinse with four 2·5-ml. portions of water. To make the solution alkaline, 15 ml. of the 30% alkali are introduced into the flask through the funnel, from a small measuring cylinder, the funnel is rinsed into the flask with not more than 1 ml. of water, and both pinchcocks are closed.

The pinchcock of the trap (*D*) is closed, and steam is at once admitted to heat the alkaline solution rapidly ; because of the vacuum jacket, only a little water condenses in the distilling flask, and the steam soon passes through the splash-trap to the condenser. All the ammonia is driven into the receiver within 3 mins. from the entry of the steam into the condenser, but as a precaution distillation is continued for 4 mins. The receiver is then lowered until the end of the condenser tube is about 2 cm. above the acid, and while the distillation is continued for another 2 mins. the condenser tube is rinsed at its lower end with 2–3 ml. of water. The flask is then removed, and the flame turned out.

Titration. The acid solution is boiled and titrated with 0·01 *N* sodium hydroxide solution until the colour changes from red to a canary-yellow, using only a very small amount of indicator in order to obtain a sharp end-point. The yellow colour should persist for 2 mins. The difference between the acid taken and that formed by back-titration corresponds with the nitrogen present as ammonia.

The apparatus is then steamed out as previously described, and the trap is drained ready for the next determination.

Calculation

1 ml. 0·01 *N* hydrochloric acid corresponds with 0·14 mgm. of nitrogen ; log 14 = $\bar{1}$·14638.

$$\log (\%\ \text{nitrogen}) = \log (\text{ml. } 0{\cdot}01\ N\ \text{HCl used}) + \log 0{\cdot}14 + 2 - \log (\text{mg. substance taken}).$$

Example :

4·372 mgm. of tyrosine used up 2·39 ml. of 0·01 *N* HCl.

Mol. wt. of tyrosine, $C_9H_{11}O_3N$ = 181·1.

$$\text{Nitrogen}\begin{cases}\text{Calculated} = 7{\cdot}73\%.\\ \text{Found} \quad = 7{\cdot}65\%.\end{cases}$$

Notes

All glass parts of new apparatus are washed first with chromic acid in strong sulphuric acid, and then with water. The rubber connexions are boiled, and then well steamed ; if moist tubing becomes dry, there is danger of leakage at connexions.

Before the first determination the apparatus is thoroughly steamed out for 30 mins., the condensed water is sucked back a few times into

the trap, and a blank test made. This gives no nitrogen value with satisfactory reagents and apparatus ; otherwise the apparatus and all reagents must be tested. Old tubing is freshly steamed out or replaced by new.

Alternative Titration Methods. With substances low in nitrogen, the titration is conveniently made iodometrically. An unsteamed 100-ml. Jena-type flask with a ground-in stopper is charged with about 5 ml. of standard 0·01 N acid, without indicator. After the distillation the solution is boiled for 3 secs. to remove carbon dioxide, and after cooling, 2 ml. of 5% potassium iodide and 2 drops of 4% potassium iodate solutions are added. After 5 mins. in the closed flask the liberated iodine is titrated back with 0·005 N sodium thiosulphate solution. The calculation is similar to that described, but the volume of thiosulphate solution used corresponding with the acid (in ml.) is divided by two before subtraction from the volume of the standard acid.

The uncertainty of the methyl red end-point and the necessity for a standardised alkali solution are eliminated by distilling into boric acid ; the resulting solution can be titrated directly with standard acid, or matched colorimetrically with the aid of a suitable indicator. The method has the further advantage that there is less chance of spoiling a determination by under-estimating the amount of acid to be added to the receiver originally ; or by the solution in the receiver being sucked back into the distillation flask. In the latter case the distillation can be continued without error. There are several modifications of the method, but that of Ma and Zuazaga [23] is recommended. In the receiver are placed about 5 ml. of 2% boric acid solution and 4 drops of a mixture of 10 ml. of bromocresol green and 2 ml. of methyl red indicators ; both indicators are 0·1% solutions of the solid dyestuffs in 95% alcohol. The colour changes from bluish-purple to bluish-green in presence of ammonia (thus indicating whether any ammonia is present, or if the receiver is contaminated with strong acid or alkali) ; when the distillation is complete, the solution is titrated with 0·01 N hydrochloric acid to a faint pink shade. The end-point is very sharp, and 8–10 distillations and titrations may be made in 1 hr. Other workers [28] eliminate titration altogether by determining the pH value of the distillate ; this, however, entails very accurate measurement of the amount of boric acid taken, and calibration against standard mixtures of boric acid and ammonia.

Direct Nesslerisation of the solution obtained after wet oxidation has the further advantage that distillation also is rendered unnecessary. The method has proved useful where speed is important, or a large number of samples have to be tested (notably for the determination of protein fractions of sera or blood plasma in clinical work [29]), but the technique should be carefully standardised, especially if the colour is matched photometrically as suggested by Levy.[30] Winkler's modification of Nessler's reagent is recommended. It is prepared by dissolving

1·0 gm. of mercuric iodide, 5·0 gm. of potassium bromide and 2·5 gm. of sodium hydroxide in 25 ml. of water, diluting to 100 ml. with water of 10° hardness, and decanting off the clear solution on the following day ; 2 ml. are added to 10 ml. of the solution to be determined, which should have been made slightly alkaline with sodium hydroxide ; the colour is matched against that produced in a similar way from a standard solution of ammonium chloride (0·03147 gm. per litre).

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DETERMINATION OF THE HALOGENS

The following excellent methods are described. The principal apparatus involved is included in the specifications issued by the American Chemical Society,[16]

1. *Combustion with Oxygen* in presence of a platinum catalyst. This is satisfactory for both halogens and sulphur, and is relatively little subject to interference by other ions. It is, however, unsuitable for very volatile substances ; in particular, those of low halogen content are liable to explode.

2. *The Carius Method.* This is a very reliable micro-version (developed by Pregl in 1910) of the standard classical procedure, in which the substance is decomposed by hot nitric acid under pressure. Its advantages are rapidity and an easily acquired technique ; its disadvantages are the possibility of explosion, and the fact that in certain

special cases unreliable (usually low) results are obtained. An additional strain on its accuracy arises from the fact that in the case of iodine the weight of the silver iodide precipitate corresponds with only about twice the weight of the iodine present. This last difficulty has, however, now been overcome by the use of Leipert's volumetric modification which is also described below.

3. *Alkalimetric Method* (Zacherl and Krainick [14]). The sample is oxidised in a current of oxygen with concentrated sulphuric acid, in presence of potassium dichromate and silver dichromate. Chlorine and bromine are thereby volatilised, whilst iodine is quantitatively retained as iodate. The two former are carried over by the oxygen into an absorption apparatus, where they react with a measured amount of sodium hydroxide solution and hydrogen peroxide according to the equation :

$$Cl_2 + 2NaOH + H_2O_2 = 2NaCl + 2H_2O + O_2.$$

The unused alkali is then back-titrated. This method is for chlorine and bromine only. It is unsuitable for liquids of low boiling-point or for very volatile solids, but satisfactory for substances containing nitrogen if the apparatus is thoroughly dried. Its great advantages, however, are those of rapidity (working time, 35 mins.) and simplicity.

4. *The Lime-Fusion Method*, which is also a classical procedure, is preferred by some workers, but as it is more suitable as a semi-micro method than as a true micro-method, it will not be dealt with here in any great detail ; it also is less suitable for iodine and bromine than for chlorine determinations. A simple bomb, consisting essentially of a steel tube which may be closed by a threaded plug, has been devised by MacNevin and Baxley,[17] and this has enabled them to extend the method to volatile liquids. The only advantage of the method is, however, its rapidity.

The Catalytic Combustion Method

Pregl's method, with slight modifications, is to be recommended. The sample is burnt in a bead tube in a stream of oxygen ; the products of combustion pass over red-hot platinum contacts, and are absorbed in sodium carbonate solution containing sodium bisulphite (to reduce any halogenate or hypophalogenate). The silver halide precipitated by adding silver nitrate and nitric acid is weighed. The method is now used only for chlorine and bromine.

Apparatus

Combustion Tubes. The decomposition of the organic substance takes place in a combustion tube of heat-resistant glass, 500–600 mm. long (Fig. 48) ; one end is drawn out to a thick-walled tip (bore, 0·5 mm.). This fine opening allows the washings used after completion of the combustion to run through only slowly, and thus lengthens the time of contact with the beads or the worm, so that these may be

rinsed quantitatively with small amounts of liquid. In front of this outlet are two constrictions in the tube, which prevent a bead from lodging in the point on washing out. The adjacent portion of the tube is filled to a length of 200 mm. with glass beads, *Ps*, about 3·5 mm. in diameter; or with a glass spiral. A constriction prevents the beads from falling out; but if it is too small it is difficult to fill the portion of the tube containing the beads with water without making an air-lock. Porcelain beads have been suggested, but they retain the products of combustion owing to their porous character and tendency to craze. Vertical combustion tubes[1, 2] and detachable absorption tubes[3] of the spiral type have been suggested as a means of avoiding removal of the combustion tube from the furnace when washing out the absorbed combustion products.

The Catalyst comprises perforated platinum cylinders (length, 70 mm.; diameter, about 7 mm.); they should be smaller than the bore of the combustion tube by only a few tenths of a millimetre (Fig. 49). Small loops of platinum welded on at

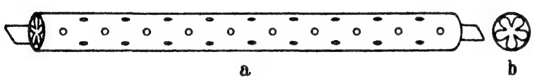

FIG. 49. Determination of halogens. (*a*) Platinum contacts comprising two concentric, perforated cylinders, with platinum loops. (*b*) Cross-section. Two-thirds actual sizes.

each end of the strengthened cylinder edges enable them to be removed from the combustion tube. In the outer cylinder are placed platinum stars, which in cross-section have the shape of a five-leaved clover. As the products of combustion, mixed with oxygen, stream slowly through two such contacts, volatile substances are completely oxidised.

Pregl made his contacts as follows: Platinum foil (50 × 15–18 mm., and 0·05 thick) is bent together longitudinally so that the cross-section resembles a Latin "Z" with somewhat lengthened initial and final horizontal strokes. Two parallel lines, 5 mm. apart, are drawn on a large sheet of paper, and the platinum foil is placed longitudinally over this so that its centre is half-way between the two lines and so that its edges extend equally on either side. Points are made bisecting the distances between the

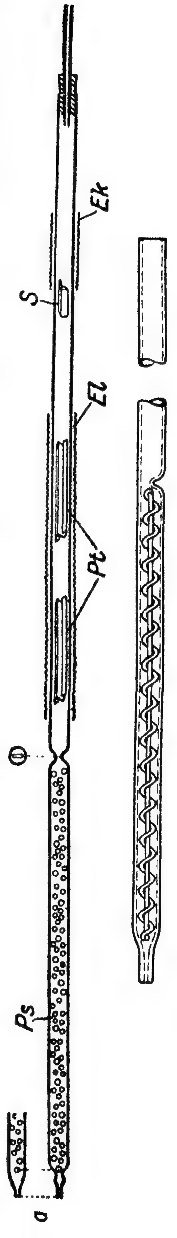

FIG. 48. Determination of halogens. (*a*) Bead tube. (*b*) Spiral tube. Half actual sizes.

parallel lines and the edges of the platinum strip. At these points and lines the foil is bent longitudinally by pressing on to it a piece of pasteboard with a straight edge, and bending its projecting portion over. Once the bends have been started they are easily completed by hand, and in spite of the thin material the contacts offer considerable resistance to distortion and bending, and are easily pushed into the combustion tube.

Platinum contacts must be boiled in dilute nitric acid and ignited well before use, and then at once placed in the bead-tube. If they lose their catalytic activity through "poisoning," they are etched with hot aqua-regia. They should always be kept in a glass dish covered with a clock glass, and should be handled only with platinum-tipped forceps.

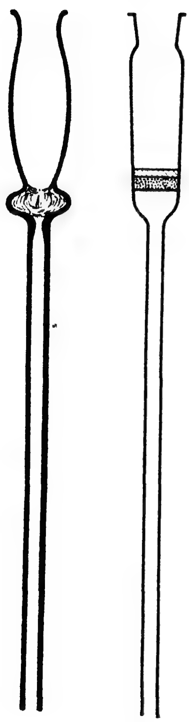

Fig. 50. Filter tubes, with (*a*) asbestos pad, (*b*) sintered glass layer. Two-thirds actual sizes.

Filter-Tubes. In 1912 Pregl used a micro-Gooch crucible for filtering the precipitates of silver halide, but later a Neubauer crucible. The desire to transfer the silver halide precipitate automatically on to the filtering surface led to the development of the filter-tube. The first filter-tubes had glass stems, on which was a spiral platinum wire supporting an asbestos filling. However, leakage of precipitates was sometimes caused by the swelling of the asbestos, and this was avoided by providing a cavity for the asbestos pad (Fig. 50, *a*). This in turn sometimes resulted in incomplete drying, and nowadays the density of filter necessary to obtain a desired and constant rate of filtration is easily obtained by means of a thin layer of asbestos, consolidated by suction on to a fritted glass base (Fig. 50, *b*).

The total length of the filter tube is 15 mm. The upper 4·5 ml. comprises a glass tube (diameter, 1 mm.) for the admission of the solution to be filtered. A fritted glass mass (Jena, 154Gl) 2–3 mm. thick, closes this part at the bottom. At about 1 cm. below the filter layer the tube is constricted conically into a shaft (length, 10 cm.; diameter, 3 mm.). The alternative Emich filter-stick is referred to on p. 26; it is recommended when the precipitate is particularly small.[4, 5]

The Suction Apparatus (Fig. 51) consists of a 250-ml. wide-necked filter flask. In the neck is a well-fitting rubber stopper, with a glass tube (length, 8 cm.; bore, 8 mm.) passing through it. On its upper end is a piece of rubber tubing (length, 20 mm.) projecting by about 10 mm. The filter-tube is passed through a small rubber stopper in this collar.

The precipitate is drawn into the filter-tube by means of a syphon, which is best made from a thin glass tube *H* (bore, 3 mm.); the long vertical arm is 20–25 cm. long, and at the top it is bent through an angle of rather more than 90°, and again after 8–10 cm., parallel to the first tube. The short arm is about 6 cm. long.

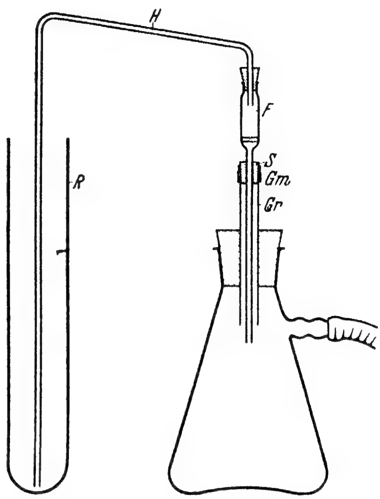

FIG. 51. Micro-filtration apparatus. Half actual size.

The Filter Tube is placed in the moistened rubber collar, a suspension of medium-fine asbestos, 2 mm. thick, is formed on the fritted glass by gentle suction, and distributed uniformly and pressed down with a sharp-edged glass rod. This operation is repeated twice, using a very fine suspension, until the compressed layer is 1·5–2·0 mm. thick. It is then well washed with water, three times with hot chromic-sulphuric acid, and again with water. Finally hot nitric acid, water and alcohol are drawn through successively. The filter-tube is dried in a regenerating block (Fig. 52) at 120° C., air being aspirated through.

The Drying Block, or Regenerating Block consists essentially of two superposed copper blocks (Fig. 52), each of which has two semi-circular channels, which together form cylindrical canals. One has a diameter of 12 mm. and serves to hold the wide portion of the filter-tube; the other has a diameter of 8 mm. The copper blocks are heated with a micro-burner having a delicate regulating-screw to control the temperature to within 2°–3° C., as shown by a thermometer inserted in a horizontal socket.

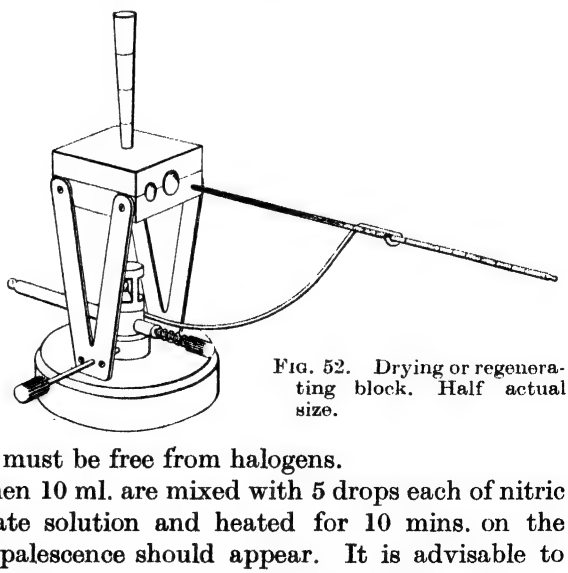

FIG. 52. Drying or regenerating block. Half actual size.

Reagents, obviously, must be free from halogens.

Distilled Water. When 10 ml. are mixed with 5 drops each of nitric acid and of silver nitrate solution and heated for 10 mins. on the boiling water-bath, no opalescence should appear. It is advisable to close the mouth of the stock bottle with a soda-lime tube.

Concentrated Nitric Acid. If this should contain chloride it is

purified by distillation *in vacuo* over silver nitrate, and stored in a brown bottle with a ground-glass stopper and glass cap.

Sodium Carbonate Solution is prepared by Reinitzer's methode.[6] About 500 gm. of commercial sodium bicarbonate are stirred up with a little water to a paste, which is drained on a Buchner funnel; this operation is repeated until the filtrate shows scarcely any chloride reaction. The washed sodium bicarbonate is added, with stirring, to about 2,000 ml. of water at 80° C. until a portion remains undissolved; after each addition there is a strong evolution of carbon dioxide. The hot solution is then filtered through a folded filter, which has previously been washed with distilled water free from halogen. The filtrate is cooled in a stream of water, and after some time the salt ($Na_2CO_3 + NaHCO_3 + H_2O$) crystallises out. After draining on a Buchner funnel and washing with a little water, 1 gm. is tested as described for distilled water; no opalescence should appear on cooling. The purified salt is carefully dried, and stored in well-stoppered wide-necked bottles, which are further protected by parchment. This salt is added to boiling distilled water in such quantity (20–25 gm. per 100 ml.) that a saturated solution is obtained. This is poured into the stock bottle while still warm, and sodium bicarbonate crystallises out on cooling. Bottles of special glass, with rubber stoppers, are now used for storing the salt, as ordinary glass is attacked.

Bisulphite Solution is prepared from the above sodium carbonate solution by slowly passing in sulphur dioxide, free from halogen, with cooling; if the temperature rises appreciable quantities of thiosulphate may be formed, and sulphur separates on acidification. The sulphur dioxide is prepared from concentrated sodium bisulphite solution by slowly adding concentrated sulphuric acid (A.R.), and passing the liberated gas through a tube containing glass wool moistened with the purified sodium carbonate solution. If test-tubes are half-filled with the sodium bisulphite solution and then sealed with a long capillary, a considerable quantity of pure solution can be stored. For use the point of the capillary is broken, and the solution is expelled, drop by drop, by warming with the hand; the point is then sealed.

The solution is tested by making 30 ml. alkaline with the sodium carbonate solution, and warming on the water-bath with 3–5 drops of hydrogen peroxide for 5 mins. After cooling, a mixture of 1–2 ml. of nitric acid, free from halogen, and 0·5 ml. of silver nitrate solution are added, and the liquid is heated for 10 mins. on the boiling water-bath. No turbidity should result.

Hydrogen Peroxide. Perhydrol grade, guaranteed free from acid (p. 100).

Silver Nitrate Solution. A 5% solution is prepared; any turbidity settles out overnight and does not affect the reagent.

Wash Liquors. Two 250-ml. wash-bottles, with ground glass joints, are required. One is filled with 96% alcohol, the other with 0·5% nitric acid.

Oxygen is supplied, preferably, from a cylinder through a needle-reducing valve.

Procedure

The combustion tube is cleaned by closing the neck with rubber tubing and a pinchcock, and pouring into the wide opening, first a little sulphuric-chromic acid mixture until the air is driven out of the spiral, and then filling up. After 15 mins. the acid is run off, and the tube is washed well with distilled water followed by alcohol or acetone, and dried in a current of warm filtered air (Fig. 53).

The substance to be analysed (4–8 mgm.) is weighed in a platinum boat as in the carbon and hydrogen determinations (p. 53). If liquids are burned in capillaries, ammonium nitrate should be used as propellant, and not potassium chlorate.

To a test-tube which is also cleaned as described, 2 ml. of the saturated sodium carbonate solution and 3 drops of the bisulphite solution are added. This mixture is aspirated slowly into that portion of the combustion tube containing the beads or spiral, so that the liquid moistens all the beads ; the liquid must not be drawn higher than necessary. The solution is now allowed to flow back into the test-tube, which it does easily if no air has been drawn in ; the remainder is then carefully blown out. After pouring away the solution the test-tube is placed over the neck of the combustion tube, and it remains there until the end of the combustion. The combustion tube is now placed on the combustion stand so that the spiral or bead layer and 4 cm. of the adjacent empty portion project beyond the combustion stand. This projecting part can be seriously distorted by heating, and it is supported by a micro-stand. The platinum contacts are boiled in nitric acid and ignited, and are placed in the tube by means of a platinum-tipped forceps and pushed in over the burner with a glass rod. In order to prevent iron particles from dropping into the tube later, its mouth is closed with a cork. The longer and shorter wire gauze rolls are then passed successively over the tube above the tube burner and about 7 cm. beyond (Fig. 48, *a*). The stopper is removed and the boat (or the capillary with a broken tip) is placed at about 5 cm. from the tube burner. The wire gauze tunnels are placed over the rolls of wire gauze. The tube is now closed by a perforated rubber stopper, fitted with a glass capillary for the introduction of oxygen. If this oxygen is supplied from a gasholder, it is convenient to introduce a wash-bottle containing saturated sodium carbonate solution, and in the rubber tubing between this and the combustion tube, a little twisted cotton-wool or a few lengths of string, to facilitate the regulation of the bubble frequency by a pinchcock to 4 ml. per min.

The platinum star contacts are first heated slowly to redness with the tube burner. Then the combustion of the substance is begun ; for this, the short roll of wire gauze is brought up to within 1 cm. of the boat, and heated in the middle with the non-luminous flame of the

moveable burner. All the precautions described for the determination of carbon and hydrogen are observed (p. 33), but the combustion must take 30 mins. because of the less active platinum contacts, and the slower absorption rate of the sodium carbonate solution. Moreover, no choking-plug is present and, therefore, substances which are heated too quickly may pass partly unburnt into the sodium carbonate solution. When the moveable burner is up as far as the tube burner, heating is continued for 2 mins. longer; then both burners are extinguished and the tube is allowed to cool in a current of oxygen.

Meanwhile the regenerating block is heated to 120° C., and the prepared filter-tube (p. 88) is placed with its stem in a moistened rubber stopper in the collar of the filtering apparatus. The vacuum is adjusted by means of a precision pinchcock (p. 35), a gentle current of air is drawn through, and the filter-tube is washed twice with water acidified with a little nitric acid and finally with alcohol. The mouth of the filter-tube is closed with an air filter, to avoid any increase in weight due to the dust particles from the laboratory air (Fig. 53); this consists of a funnel filled with cotton-wool, in a cork in the filter-tube. After washing, the filter-tube is taken from the rubber stopper, wiped on the outside with a towel, the stem placed in a tight-fitting rubber tube, and the tube dried at 120° C. for 5 mins. in the regenerating block, air being drawn through as before; the stem is finally placed in the narrow hole to remove the last traces of moisture. The dried filter-tube is taken from the pump and, after removal of the air filter, wiped; it must always be mouth upwards to avoid loss of the silver halide precipitate. It is wiped first with moist flannel and then with chamois leather (as described on p. 52), then placed on a stand next to the balance, allowed to cool for 15 mins., and weighed to 0·005 mgm. 5 mins. later, using a counterpoise (p. 13).

FIG. 53. Air filter.

Filter-tubes need not be cleaned out between determinations, but when they contain 60–80 mgm. of silver halide a slackening of the velocity of filtration becomes noticeable, and it is advisable to dissolve the precipitate in warm potassium cyanide solution and to clean the tube as already described.

The boat and the platinum contacts are now withdrawn from the cool combustion tube with a platinum wire hook. If a residue is visible in the boat under a lens, its weight must be determined. Before the roll of wire gauze is removed, a cork is again inserted in the mouth of the tube, and particles of iron from the roll are subsequently removed carefully from the tube with a cloth. The test-tube is held over the neck of the tube, which is clamped in a stand at an angle so that the test-tube stands upon the bench and about 4 cm. of the combustion tube are in the test-tube. The cork is now removed, 2–3 drops of bisulphite solution are placed in the tube, and the beads or the spiral tubes are completely covered with water in a continuous

stream from the wash-bottle. With bead-tubes air-locks sometimes occur, but on rotating the tube the bubbles escape. After running off the first washings, the tube is rinsed twice more with the same amount of acidified water, and finally the neck is rinsed.

The collected washings are treated with 2 drops of perhydrol, to oxidise the sulphite, and are then heated in a boiling water-bath for 5 mins., a beaker being inverted over the test-tube to protect it. A mixture of 1 ml. of concentrated nitric acid and 2 ml. of silver nitrate solution is added to the hot solution ; an opalescent cloudiness results. After heating again for 10–15 mins. in the boiling water-bath, precipitation is quantitative and the clotted silver halide may be filtered from the cool solution.

The weighed filter-tube is placed in the filter-flask. The syphon, cleaned before each series of determinations as already described, is placed in a rubber stopper which exactly fits in the mouth of the tube, so that the syphon ends 2 cm. below it (Fig. 51). The stopper is moistened with distilled water to obtain an air-tight connexion. The filtered solution should drop on to the asbestos without touching the wall of the filter-tube. The long limb of the syphon is lowered until it is just above the precipitate in the test-tube, and the pump is turned on carefully until about 2 drops of filtrate pass per second. After most of the liquid has been removed, the precipitate is shaken well with the dilute acid, and then syphoned off. If particles still adhere to the walls, they are rinsed to the bottom of the test-tube, so far as possible, with a fine stream from the wash-bottle. The walls of the test-tube are now rinsed with alcohol ; this removes the last particles of silver halide by reason of the surface tension effects at the junction of the alcohol and water. The alternate rinsing with acidified water and alcohol is repeated twice. It is necessary to loosen adherent particles of the precipitate with the feather (see p. 102) only in exceptional cases.

When filtration is finished the rubber stopper with the syphon is carefully removed from the filter-tube, the portion of the syphon within the tube is rinsed with alcohol, and the filter-tube is filled completely with alcohol. After the alcohol has run through, the air filter is put on. The drying and weighing of the tube and precipitate are carried out as described on p. 92.

Calculation

1 mgm. Cl corresponds with 4·043 mgm. AgCl.

1 mgm. Br corresponds with 2·348 mgm. AgBr.

log (% halogen) = log (mgm. silver halide) + log (factor) + 2 − log (mgm. sample)

	Factor	Log (factor)
$\frac{Cl}{AgCl}$ . .	0·2474	$\bar{1}$·39334
$\frac{Br}{AgBr}$ . .	0·4255	$\bar{1}$·62894

Example : 3·615 mgm. chlorobenzoic acid yield 3·33 mgm. AgCl.
Mol. wt. of $C_7H_5O_2Cl$ = 156·54
Theory, 22·66% Cl.
Found, 22·79% Cl.

Volumetric Determination of Iodine (Leipert's Method [7])

Free iodine liberated in Pregl's spiral or bead-tube is collected in dilute sodium hydroxide solution, and oxidised with bromine water to iodic acid ; Leipert originally removed excess of bromine by passing in steam. Potassium iodide is added to the iodic acid formed, and the liberated iodine is titrated with 0·02 *N* sodium thiosulphate solution. The advantages of this procedure are rapidity and the high multiplying factor (see p. 86). Bromine analogously may be oxidised to bromate with chlorine water, and determined similarly, even when present with chlorine and in small proportions.

Reagents are as follows :—

Sodium Hydroxide Solution. Five grams of sodium hydroxide (tablet form) are dissolved in 100 ml. of water, 50 ml. of which should not consume any sodium thiosulphate solution in a blank titration.

Sodium Acetate Solution is made by dissolving 40 gm. of sodium acetate (+ $3H_2O$), A.R., in 200 ml. of water (see above).

Sodium Acetate in Glacial Acetic Acid. Ten gm. of sodium acetate (+ $3H_2O$) are dissolved in 100 gm. of glacial acetic acid.

Bromine, free from iodine, is kept in a dropping-bottle.

Formic Acid (80–100%) is stored in a dropping-bottle or dropping-pipette.

Potassium Iodide (free from iodate) ; a 10% solution in pure water (see above) is used.

Sodium Thiosulphate. Fifty millilitres of 0·1 *N* thiosulphate are run into a 250-ml. graduated flask from a graduated pipette, 1·5 ml. of amyl alcohol are added and the flask is filled up to the mark. The factor for the solution is determined with 0·05 *N* potassium dichromate, as in the determination of *iso*propylidene groups (p. 170), using a micro-burette.

2 *N Sulphuric Acid.*

Starch Solution, prepared as described on p. 173.

Methyl Red, prepared as described on p. 81.

Procedure. Into the upper edge of the cleaned and dried spiral or bead-tube (p. 86) are aspirated 4–5 ml. of 5% sodium hydroxide solution from a test-tube. The solution is then allowed to run out, and the rinsed test-tube is placed over the end of the tube. The combustion is then carried out as described on p. 91, using the platinum star contacts and 3–6 mgm. of the substance weighed in a boat or in a capillary. Oxygen passes through the wash-bottle containing sodium carbonate at the rate of 3 ml. per min. Combustion should be finished in 25–30 mins., but care must be taken that, particularly during the decomposition, no iodine should separate

behind the moveable burner; if it does it must be driven back again by careful heating. Iodine separating on the cooled wall of the tube between the tube burner and spiral is also driven into the absorbing solution by careful heating, and while the tube cools in a stream of oxygen, the weighing for the next determination is made.

Five millilitres of the sodium acetate solution are pipetted into a 100-ml. glass-stoppered conical flask. Then 4 ml. of 10% sodium acetate solution in glacial acetic acid containing 2–3 drops of bromine are poured into a test-tube, and aspirated from it into the combustion tube to 1–2 cm. above the spiral or bead layer. The mouth of the tube is closed with the finger, the neck is placed in the conical flask, and the tube is then clamped at 60°–70° to the horizontal and the solution allowed to flow out; 6 ml. of water from the test-tube are then also aspirated through and run into the conical flask. Finally, the mouth of the combustion tube is rinsed twice with 6–8 ml. of water from a wash-bottle while the tube is rotated.

Before the titration the bromine must be destroyed by allowing 2–3 drops of formic acid to run down the wall of the flask, with careful shaking. If the solution is decolorised it is first tested for free bromine by the smell, and then by adding a very small drop of methyl red with a glass thread. If the indicator is decolorised bromine is still present, and 1 drop more of formic acid must be added. If the solution remains slightly pink, 2 ml. of the 10% potassium iodide solution and 5 ml. of 2 *N* sulphuric acid are added. After 5 mins. with the flask stoppered, the iodine is titrated rapidly with 0·02 *N* thiosulphate solution, to a slightly yellow colour, 4–6 drops of starch solution are added, and the solution is titrated to a slightly pink end-point, using methyl red as indicator.

Calculation. 1 ml. of 0·02 *N* thiosulphate is equivalent to 0·4231 mgm. of iodine; log 0·4231 $= \bar{1}\cdot62644$.

log (% I) = log (ml. 0·02 *N* $Na_2S_2O_3$ used) + log 0·4231 + 2 − log (mgm. substance)

Example:

3·572 mgm. iodobenzoic acid are equivalent to 4·32 ml. 0·02 *N* $Na_2S_2O_3$

Mol. wt. of $C_7H_5O_2I$ = 248·00 $\begin{cases}\text{Theory, } 51\cdot19\% \text{ iodine.}\\ \text{Found, } 51\cdot18\% \text{ iodine.}\end{cases}$

Because 1 atom of iodine in the substance yields 6 atoms for titration, with carefully prepared solutions very accurate results are obtained which seldom deviate from the theoretical by more than ± 0·2%. With analytically pure reagents and twice-distilled water no blank determination is necessary. The method has been proved in various laboratories.[8]

Kirk and Dod [9] have claimed slight improvements on the above method by using a mixture of 2 ml. of saturated sodium carbonate solution and 3 drops of strong sodium bisulphite solution (chloride-free) as the absorbing agent; and by neutralising with 2 ml. of glacial acetic acid instead of with sulphuric acid. Most of the excess of bromine

is removed by boiling the solution after it has become uniformly brown (to avoid loss of iodine), and this procedure also removes some of the water, and so sharpens the end-point. The last traces of bromine are removed by adding a little phenol, the solution is acidified with 2 ml. of 10 *N* sulphuric acid, and the iodide is added. Errors quoted, however, average about 0·3–0·5%.

The Micro-Carius Method

Apparatus

The Pressure Tubes in which the sample is heated with nitric acid should be 10–12 mm. internal diameter and 1·0–1·3 mm. thick, and made of hard glass (*e.g.*, Jena 20) ; if they are 250 mm. long they serve for a number of analyses. Before use, the tube is filled with sulphuric-chromic acid mixture, placed in a tall beaker in a boiling water-bath for 15 mins., washed with distilled water, and dried at 115° C. ; thorough drying is important because of the technique used for weighing the substance (see below).

The Furnace. The tube can be heated in any tube furnace. Pregl recommended Haack's furnace, which has the advantages that the necessary temperature can be attained rapidly, and that the furnace can be quickly cooled again. Temperature control is easier if it is placed in a small fume cupboard, with a strong board in front of the small sliding windows as a protection. With electrically heated furnaces it is advisable to insert a clock-mechanism in the circuit to cut off the current automatically after 5 hrs. Pressure tubes can then be placed in the furnace in the evening and be found cool next morning. Kuck and Griffel [10] describe a " home-made " electric furnace which heats up to 250° C. and cools down from 300° C. in about 15 mins.

Procedure

Weighing. Roth recommends that solid substances (3–8 mgm.) should be inserted as far as possible into the pressure tube by means of weighing tubes with long handles (see p. 73) ; particles adhering to the wall are removed by tapping with a wooden rod. Substances which evolve halogen even in contact with cold nitric acid are weighed in a hard glass tube, 15 mm. long and 3 mm. in diameter, sealed at one end. After charging the pressure tube with silver nitrate and nitric acid, this tube is pushed down into it.

Micro-weighing bottles, which are easily prepared from hard glass tubes, are particularly suitable for weighing liquids (Fig. 54). If a ground-in stopper is used, substances having a slight vapour pressure may be so weighed. If, however, the liquid is more volatile, it must be weighed by Pirsch's method (p. 202) ; the handle of the capillary is then a glass rod, 10–15 mm. long and 3 mm. in diameter. The capillary filled with the substance is placed with its thin end in the bottom of the charged pressure tube, which is immediately sealed. To break the capillary the cooled tube is held point upwards in one hand

and sharply hit from below a few times with the other hand; the capillary is thus broken by the heavy glass rod. It is obvious that the precipitate will be mixed with fine splinters of glass; it must, therefore, be weighed in a freshly prepared filter-tube, and this must be re-weighed after dissolving out the silver halide in potassium cyanide solution. With very volatile liquids, careful combustion in the bead-tube is preferable.

Oils and *liquids of a syrupy consistency* are introduced into the boat with a thin glass rod or weighed in a small weighing-bottle (Fig. 54).

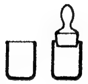

FIG. 54. Micro-weighing bottles. Actual sizes.

Decomposition. Silver nitrate (10–20 mgm.) is added to the sample, and 0·5–0·8 ml. of halogen-free concentrated nitric acid are introduced from a fine pipette, the tip of which is about 5 cm. inside the slanting pressure tube which is rotated so that any particles are washed down. A thick glass rod and the open end of the charged pressure tube are pre-heated in the luminous blowpipe flame. The glass is then sealed to the inner edge of the tube in the hottest part of the non-luminous flame, 2–3 cm. below its mouth, rotating slowly until the glass begins to fuse; it must not be drawn out. When the walls have almost closed, the point of fusion is drawn out to a thin-walled capillary in the outer part of the flame, and sealed; the sealed end is annealed in a luminous flame.

Organic substances are usually decomposed by heating at 280° C. for 5 hrs.; 300° C. is better for aromatic halogens. After cooling to room temperature, a length of about 50 mm. of the tube, in its slanting position, is withdrawn from the oven. The nitric acid is driven from the capillary and the upper part by touching these with the non-luminous flame of a Bunsen burner. The point is then softened with this flame until it opens under the slight inside pressure. A scratch 1 cm. long is made in the opened tube with a glass-cutter, at 1 cm. below the constriction, and the scratch is broken with a red-hot drop of glass. To prevent particles of glass from falling into the nitric acid, the tube is held almost horizontally, the broken point is carefully removed, the sharp edge rounded in the blowpipe flame, and the tube softened to a distance of 2–3 cm. down, so that it takes up any adherent splinters. This precaution is most essential, because high halogen values are often due to glass splinters in the precipitate. This, in fact, is the most frequent source of error in the method, and various modifications in technique have been suggested to overcome it.[11]

Thus, Unterzaucher and Röscheisen [12] proceed as follows: The cooled Carius tubes while still in the furnace are next opened by softening the end of the capillary, so as to blow off the pressure, and are afterwards closed again by sealing in the same place with a special blowpipe flame. A fine-pointed oxygen flame, 2–3 cm. long, is produced by leading oxygen at the required rate into a gas flame, the Carius tube is warmed (with constant rotation) and then brought tangentially into

contact with the blue cone of the oxygen flame. The rotating tube is gradually brought further into the oxygen flame, and when a ring-shaped softening of the glass occurs, rotation is stopped and part of the ring is heated directly with the pointed flame, until the inside pressure developed bursts a small oval opening in the wall of the tube (see Fig. 55 ; *a*, front view ; *b*, side view). The flame is now directed on to the oval opening, and the tube is simultaneously rotated and bent backwards (*c*) so that the widening of the opening forms a cut around the whole tube (*d*). Dark goggles should be worn because of the brilliance of the flame.

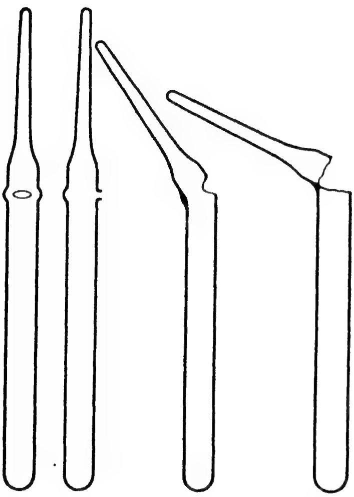

FIG. 55. Stages in the method of opening a pressure tube without splintering.

The sides of the tube are rinsed down with 2 ml. of acidified water, with rotation. If the precipitate is large it may collect into a ball which may occlude silver nitrate, and to avoid this error the precipitate is crushed in the acid with a clean rounded-off glass rod and rinsed in the tube with acidified water. If the substance is to be weighed in the porcelain boat or in a weighing-bottle, it is drawn to the mouth of the tube with the platinum hook, held with platinum-tipped forceps and rinsed thoroughly into the tube ; the nitric acid is diluted to about 3 ml. with water, and the tube is placed on the boiling water-bath for about 5 mins. in a beaker. Immediately the solution is cool it is filtered (p. 93), avoiding unnecessary exposure to light.

Calculation. See p. 93.

If the silver halide precipitate is collected in a Neubauer micro-crucible (p. 102) and heated at 300° C. with an excess of dry, pure ammonium bromide or iodide, it is transformed into silver bromide or iodide, respectively, the remainder of the ammonium salt being lost by volatilisation. Moser and Miksch [13] have used this method to determine mixtures of these two halides.

The Alkalimetric Method [14]

Apparatus (Fig. 56)

The Oxidation Vessel is 100 mm. long ; its neck is 80 mm. long and 10 mm. internal diameter. The bulb is heart-shaped, and 18 mm. in diameter at the widest part. The ground-in glass stopper is held fast in the neck, to a depth of 20 mm., by hooks and steel springs (*S*). Through it passes an inlet tube (length, 90 ; diameter, 2 mm.) ending 4 mm. from the bottom of the oxidation flask. Above the joint the inlet tube is connected with the dropping funnel *T* (capacity, 4–5 ml.)

by a stop-cock H_1 having a ground-in inlet tube (diameter, 8 mm.) bent at right angles at the top ; this also is held by steel springs. The delivery tube to the absorption apparatus branches off immediately above the joint ; after 5 cm. it is bent downwards at right angles and slightly constricted at the end. The vertical tube is about 27 cm. long and 2 mm. internal diameter.

The Absorption Apparatus is a glass tube (diameter, 12 mm.) which widens into a funnel at the top. At the lower end is a well-fitting inclined stopcock H_2, and the tube ends in a thick-walled jet 4 cm. long and 1–2 mm. more. The central inlet tube is closely surrounded by a glass spiral, which reaches to the bottom of the funnel and touches the wall of the outer tube. This compels the bubbles of gas which escape at the tip of the inner tube, to rise with a circular motion, and so to remain in contact with the solution as long as possible.

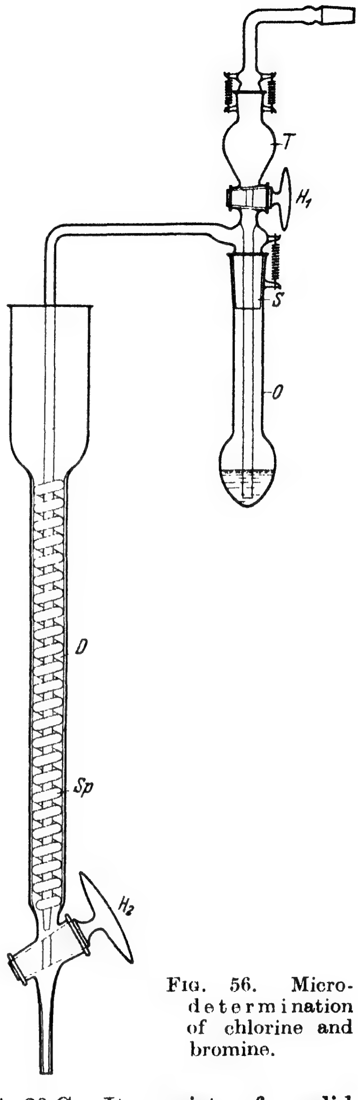

FIG. 56. Micro-determination of chlorine and bromine.

The Aluminium Heating Block. Halogen determinations are usually carried out in the same room as carbon and hydrogen determinations. It is therefore obvious that organic vapours from baths of oil or paraffin must be avoided so far as possible.

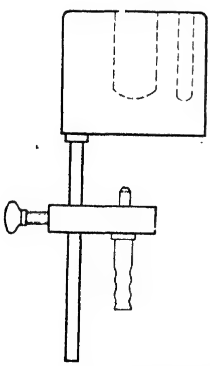

FIG. 57. Aluminium heating block with micro-burner. Half actual size.

The aluminium block with micro-burner (Fig. 57) enables the temperature to be controlled to within $\pm$ 2° C. It consists of a solid aluminium cylinder (diameter, 80 ; height, 70 mm.), clamped in a stand by means of a screw-on brass rod. The central cylindrical hole (bore, 24 mm.) serves for the admission of the oxidation flask, whilst the thermometer is placed in the narrow, 8-mm. hole. The micro-burner may be screwed fast to the vertical brass rod at any desired height ; its flame is regulated by a pinchcock on the tubing.

Reagents

Concentrated Sulphuric Acid (*d*, 1·84). A.R. grade.

A mixture of equal parts by weight of **potassium dichromate, A.R.,** and **silver dichromate.** The silver dichromate is best prepared [15] by boiling 10 gm. of silver nitrate and 6 gm. of chromic acid (A.R.) with 1 litre of distilled water until all is dissolved. After a few hours the silver dichromate begins to separate in dark brown, glittering crystals. On the next day the supernatant liquid is poured off, the crystals are washed twice on a Buchner funnel with distilled water, dried over phosphorus pentoxide in a desiccator, and dried and powdered and stored in a brown wide-necked bottle.

Hydrogen Peroxide (Perhydrol, " acid-free "). Even this grade has a slightly acid reaction, and the acidity must be determined on the contents of every new bottle ; 1 ml. is mixed with 1–2 ml. of 0·01 *N* hydrochloric acid, boiled, and titrated with 0·01 *N* sodium hydroxide solution, using methyl red as indicator.

Standard 0·01 *N* **Hydrochloric Acid** and **Standard** 0·01 *N* **Sodium Hydroxide Solution,** in automatic micro-burettes (p. 29).

Methyl red is prepared as described on p. 81.

Procedure

After cleaning the apparatus with sulphuric-chromic acid and distilled water and drying thoroughly in the oven, the ground-in attachment is clamped in a cork with the delivery tube horizontal, and the aluminium block is heated to 115°–120° C. For the chlorine determination, 4–6 mgm., and for the bromine determination, 5–8 mgm., of the substance to be analysed are placed on the bottom of the flask, using the weighing tubes with long handles. Liquids of low boiling point are best weighed in the very small weighing bottle (p. 97).

To the weighed material are added about 0·5 gm. of the dichromate mixture. The joint for the stopper is moistened with concentrated sulphuric acid, and the flask is closed, and fastened. Two ml. of concentrated sulphuric acid are placed in the funnel with the cock H_1 closed ; the inlet tube is inserted and fixed, 1 ml. of peroxide is pipetted into the absorption apparatus and about 7·5 ml. of 0·01 *N* sodium hydroxide solution are added from the burette.

The oxygen is drawn from a cylinder through a needle reducing-valve into a wash-bottle containing sodium carbonate solution, and the bubble-frequency is permanently adjusted by means of a Mariotte flask (p. 41), so that 8 ml. pass per min. The wash-bottle is connected with the inlet tube of the funnel, and on opening the cock H_1, the oxygen forces the sulphuric acid into the flask for the oxidation. The flask is then placed in the hole of the heating-block for 30 mins. During this time the weighing for the next determination may be made. The cock H_1 is then closed and the oxygen supply disconnected. A 100-ml. silica flask is then placed under the outlet of the absorption apparatus, the contents of which are washed into it with three 4-ml.

portions of water. For the titration, a small drop of methyl red is added with a glass thread, and 0·01 *N* acid is run in until a distinctly acid reaction results. The carbon dioxide liberated is then boiled off, another drop of methyl red is added, and the solution is titrated with 0·01 *N* sodium hydroxide solution to a canary yellow colour.

Calculation

From the total volume of 0·01 *N* alkali used in the receiver and for the titration are subtracted the volumes of 0·01 *N* acid required for the perhydrol blank test and for acidification. One millilitre of 0·01 *N* sodium hydroxide solution is equivalent to 0·3546 mgm. of chlorine, or to 0·7992 mgm. of bromine.

$$\log(\%\ \text{halogen}) = \log(\text{atomic wt. of halogen}) + \log(\text{ml. } 0{\cdot}01\ N\ \text{NaOH}) - \log(\text{mgm. sample used}).$$

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DETERMINATION OF SULPHUR

Sulphur in organic substances is determined by converting it into sulphuric acid, or a sulphate, which is determined gravimetrically or (preferably) volumetrically. There are two methods of oxidising the sulphur :—

1. By combustion in the bead-tube (p. 87), the products of combustion being absorbed in hydrogen peroxide which oxidises any sulphurous acid to sulphuric acid.

2. By Pregl's modification of the Carius method, in which decomposition is carried out in the pressure tube with concentrated nitric acid in presence of a crystal of barium chloride. In the gravimetric method the sulphate is precipitated as barium sulphate and weighed ; there are several alternative volumetric methods, and the best of these are described below (p. 107).

Pregl's Bead-tube Method

Apparatus

The Combustion Apparatus is the same as is used for the halogen determination (p. 86) ; electrical heating is preferable, as high results

have sometimes been found when gas burners are used.[1] Additional apparatus is described below, and also has been the subject of specifications issued by the American Chemical Society.[2]

The Micro-Neubauer Crucible in which the barium sulphate precipitate is collected, is fitted with a lid and has a capsule fitting over the base; it is 14 mm. high and has a diameter of 12 mm. at the top and 10 mm. at the base. The filtering layer is compressed iridio-platinum sponge, which completely retains barium sulphate precipitated in the cold and yet has a comparatively high filtration-rate; careful experiments showed that a satisfactory crucible passes on an average of 4 ml. of filtrate per min. on suction with the mouth. The crucible specified by the American Chemical Society [2] is 35 mm. high (external diameter, 30 mm. at the top and 23 mm. at the base) and is glazed black inside; the base and walls are 2 and 1 mm. thick, respectively.

Before every determination, the inside of the crucible is cleaned under the tap with a small wad of cotton-wool wound round a match (not wire); it is then repeatedly washed with water while in the rubber collar of the filtering apparatus (p. 103). Very occasionally only, and after long use, it is necessary to remove particles of barium sulphate held in the filtering layer by means of a little warm concentrated sulphuric acid. After cleaning the crucible in this manner it is advisable to filter on it a freshly prepared barium sulphate precipitate, and to wash this thoroughly to ensure that the pores of the filter bed are properly closed. Neubauer crucibles with filter-beds of porous porcelain may be used, but only if the glaze extends beyond the rounded lower edge of the crucible, and not merely over its outer surface. If the lower edge and the adjacent parts are rough and unglazed, particles of the crucible may be scraped off when it is pressed into the rubber collar, and particles of rubber may be retained in the rough porcelain wall.

FIG. 58. The "feather." Half actual size.

The "Feather." The transfer of the precipitate from the dish to the crucible is aided by the use of a stiff feather. Fig. 58 shows a snipe feather cemented into a glass tube (length, 120–150 mm.; external diameter, 2·0–2·5 mm.) sealed at one end. A suitable cement is described on p. 45; a small piece of it is stuck in the open end of the capillary and melted by warming on the regenerating block, and the shaft of the feather is pushed slowly into the warm glass tube. After removing mechanically the cement which adheres to the outside, the feather is washed quickly and successively in benzene, alcohol and ammoniacal soap solution, by carefully rubbing it between the fingers. It should always be kept in a stoppered test-tube.

The Filtration Apparatus used for the halogen determination (p. 89) is suitable. The crucible, T, is inserted in the rubber sleeve, M (Fig. 59). Filtration must be carried out with slight suction only (*e.g.*, with the mouth) through a rubber tube, 50–60 cm. long, which is provided with a pinchcock. If the pump is used a glass stopcock, round each end of the barrel of which fine grooves are scratched, is inserted in the pressure tubing, between it and the filter-flask. Regulation with pinchcocks should be carried out with care because it may easily lead to a sudden breaking of the vacuum.

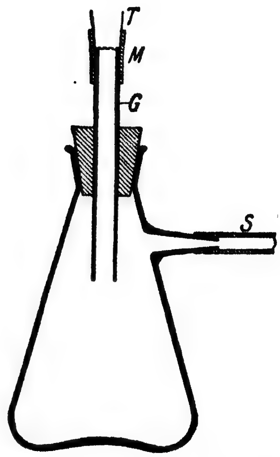

FIG. 59. Micro-filtration apparatus for barium sulphate. Half actual size.

Fig. 60 shows Wintersteiner's automatic filtration apparatus.[3] The micro-Neubauer crucible, T, is attached to a glass tube (external diameter, 12·5 mm.) inserted in a small filter-flask in the usual manner by means of the rubber collar. The glass cover, A, is placed over the crucible after slightly moistening the rubber collar. The shorter limb of the capillary syphon tube is inserted into the upper end of this cover in such a manner that fairly large drops can form at its widened lower end (S), which should be at about half-way down the crucible. A side-tube (which is inclined downwards in some apparatus [2]) carries a rubber tube, II, about 50

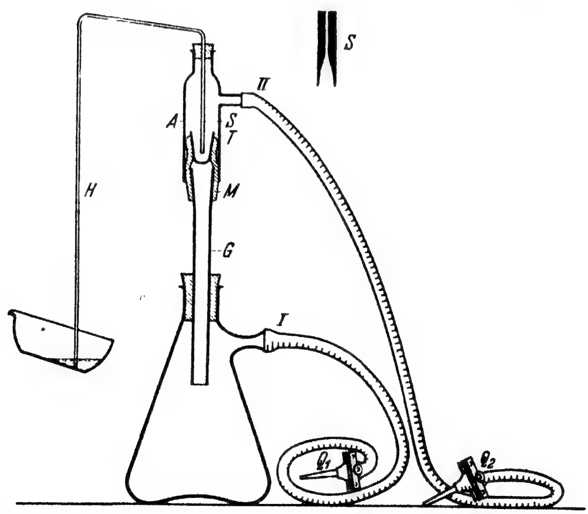

FIG. 60. Automatic micro-filtration of barium sulphate.

cm. long, with a glass mouthpiece and pinchcock, Q_2, while the side-tube of the filter-flask, I, is provided for similarly. If the filter-bed of the crucible is initially dry and suction is applied at Q_1 the vacuum extends through the filter-bed into the glass cover, and the suspension

of barium sulphate is aspirated through the syphon and on to the filter-bed; this proceeds smoothly so long as the filter-bed of the crucible is covered by liquid. If the pressure rises in the cover, A, sufficient liquid is no longer transferred; the reduced pressure in the filter flask can no longer extend to the cover, as the now moist filter-bed offers too great a resistance. Careful suction with the mouth at Q_2 then restarts the transfer of the liquid, and filtration recommences. Suction must not be too strong, or the liquid may creep over the edge of the crucible.

The last traces of precipitate are transferred to the crucible in the usual manner by rinsing the dish alternately with alcohol and with water acidified with hydrochloric acid. The pinchcock, Q_2, is now opened, the glass cover removed, the syphon detached, and the shorter limb of the syphon rinsed well into the crucible.

Procedure

Combustion is carried out in the tube containing beads or spirals, as in Pregl's method for chlorine and bromine (p. 86). Only essential differences, therefore, will be discussed here.

The weight of sample should be 4–7 mgm. The bead-tube is filled, by suction, with 10 ml. of 20% perhydrol. This is stable for 1 day. The combustion tube is placed on the stand, the end is covered with a clean test-tube, and the tube is charged; a bubble velocity of 3 ml. per min. is set up, and the combustion is carried out as described on p. 91. The moveable burner must be moved forward with special care, because the quantitative absorption of the sulphur trioxide in aqueous solution is rather slow. Combustion takes 30–40 mins. More material must be used if the substance contains little sulphur, and the time of combustion should then be increased to at least 1 hr.

After the tube has cooled in oxygen and the boat and the platinum stars have been removed, the tube is clamped at an angle (*cf.* p. 92), and its contents are rinsed into a glass or platinum dish (diameter, 7 cm.). New glass dishes must be steamed for several hours, or high values may be obtained, owing to the separation of silica.

The mouth of the combustion tube is rinsed three times with 1% (by vol.) hydrochloric acid, using as much acid each time as will cover the entire spiral or bead layer. The test-tube which was placed over the neck of the combustion tube is also rinsed.

Precipitation. To the wash-waters in the dish, one or two 20-mgm. crystals of barium chloride are added; the solution is covered with a clock-glass, convex side downwards, and evaporated on the water-bath. At first, decomposition of the perhydrol is observed, and during this period the precipitation of the barium sulphate begins. It is desirable to obtain as coarse a precipitate as possible; evaporation is therefore taken to dryness, and the residue is dissolved in a little water and then filtered. If the sulphur content is relatively high, the addition of about 1 ml. of a saturated solution of picric acid for every 10 ml. of

solution precipitated, before the barium chloride is added, reduces the interval before filtration and renders evaporation unnecessary.

Preparing the Crucible. During the evaporation the cleaned crucible (p. 102), without its lower cover, is placed in the moistened collar of the filtration apparatus (Fig. 60), so that it rests on the glass tube, *G*. It is washed well with the 1% hydrochloric acid, removed from the collar with three fingers and closed with the lid and lower cap and placed on the lid of a large platinum crucible (diameter, 30 mm.) on a silica triangle. It is first dried with a small flame, during which operation it often oscillates because of the formation of steam. As soon as it is dry, it is heated to redness for 3 mins. with a stronger flame. The lid may also be heated to redness in the flame for a short period ; it is held with platinum-tipped forceps. The flame is turned out, the crucible is allowed to cool to about 150° C., and it is then placed on a nearby copper block and transferred, with this, to the desiccator near the balance.

The working-time may be shortened by transferring the crucible to a second copper block after cooling for a few minutes ; the crucible may then be ignited and weighed. The crucible (with its lid and lower cap) is finally transferred to the balance with platinum-tipped forceps ; it is held in the centre without much pressure.

Filtration. The weighed crucible is returned to the copper block in the desiccator, and carried in it to the filtration apparatus. The lid and lower cap are removed and placed on the copper block, and the crucible is fitted into the moistened rubber collar of the filtration apparatus.

In the meantime, the barium sulphate precipitate has settled on the bottom of the dish. In order to transfer it to the crucible the dish is held in the left hand, while the right holds the feather vertically over the middle of the crucible. The clear solution is then poured down the feather into the crucible, without disturbing the precipitate, until the crucible is full. The liquid is sucked through, fresh solution being added when it has almost all gone through. It is advantageous, during this somewhat delicate operation, to rub the outer edge of the dish, at the point of pouring, with a slightly greased finger, and to hold both elbows close to the body, so that the edge of the dish and the feather are always in contact above the centre of the mouth of the crucible. The top of the liquid in the crucible should never be touched with the point of the feather, as the precipitate already in the crucible will creep up it.

After the liquid has been decanted off, the dish is rinsed with 1–2 ml. of the dilute hydrochloric acid ; the precipitate is stirred up with the point of the feather and at once transferred to the empty crucible. The dish is rinsed with water, then rubbed with the feather from the edge to the centre, and the contents once more poured into the crucible. The whole of the inner surface of the dish is then rinsed with alcohol in a continuous stream, and the liquid once more transferred

to the crucible down the feather. The crucible is then rinsed as before with water and the transfer of the last, almost invisible, particles of precipitate is assisted by rubbing with the feather. This rinsing with alcohol and water alternately is repeated at least once more, and the last residues are thus removed by using twice the surface tension between alcohol and water; however, the operation requires practice. It is also necessary to use water for the final washing; if alcohol is present violent spiriting may occur on subsequent heating. Alternatively, Wintersteiner's method (p. 103) may be used.

The crucible is finally washed twice more with acidified water, the lid and lower cap are replaced, and it is ignited (p. 105) and cooled. In order to remove any barium chloride carried down with the precipitate, the crucible is replaced, after ignition, in the moistened rubber collar, washed three times with the dilute acid, ignited as before with the lid and lower cap in position, and weighed to 0·005 mgm. A second wash and ignition should result in a loss in weight of not more than 0·005 mgm. Saschek [5] finds that drying at 150° C. is as effective as ignition.

Calculation

log (% S) = log (wt. $BaSO_4$) + log (factor) + 2 − log (wt. sample).
1 mgm. S is equivalent to 7·281 mgm. $BaSO_4$.

Factor $\frac{S}{BaSO_4} = 0{\cdot}1373$. Log (factor) $= \bar{1}{\cdot}13770$.

Example:

Sulphonal.	Barium sulphate	Percentage of S found.
6·1785 mgm.	12·745 mgm.	28·31

Molecular weight of sulphonal, $C_7H_{16}O_4S_2$ = 228·29.
Theoretical S content = 28·09%.

The Pregl-Carius Method

Pregl found that if organic material is decomposed with concentrated nitric acid without the addition of barium chloride, slight losses in sulphur occur on opening the tube. If the pressure-tube is heated at 280°–300° C. for 5 hrs. with concentrated nitric acid, in presence of 10–20 mgm. of barium chloride (which is powdered to avoid occlusion), losses still occur frequently even with glass of good quality. This is ascribed to the fusion of barium sulphate into the glass, so that it cannot be removed with the feather. Pregl has also drawn attention to the high values due to separation of silica from the glass, which occurs even when a solution of barium chloride is concentrated in a glass dish. The following precautions should therefore be observed[6]:

1. New Jena (red-streak) or similar glass pressure-tubes must always be used.
2. The temperature should not rise above 270° C.
3. Not more than 15 mgm. of barium chloride should be added.

In other respects the procedure is as described on p. 104.

The above method may be combined with the halogen determination by decomposing the sample in the pressure-tube with nitric acid and a crystal of silver nitrate. The filtrate and washings from the silver halide precipitate are collected in a short test-tube placed in the filter-flask, and rinsed quantitatively into a Jena-type glass dish, and the sulphuric acid is precipitated on the water-bath with 2–3 ml. of a 1% solution of barium nitrate. If the reagent contains halogen (silver nitrate test) it should be precipitated from the warm solution with a little silver nitrate, and the filtrate used. The barium sulphate precipitate is dealt with as described on p. 105.

Volumetric Methods

In Absence of Nitrogen and Halogens. The products of combustion in the bead-tubes are absorbed in 20% perhydrol solution, which has been neutralised with 0·01 *N* sodium hydroxide to a canary-yellow colour, using methyl red as indicator. The bead-tube is rinsed (p. 104) with distilled water, the washing-water is collected in a 100-ml. wide-necked quartz flask, a small drop of methyl red is added from a glass thread, and the sulphuric acid formed is titrated with 0·01 *N* alkali until a canary-yellow colour is maintained for 2 mins. without any change to pink.

One millilitre of 0·01 *N* sodium hydroxide solution corresponds with 0·1603 mgm. of sulphur ; log factor = $\bar{1}$·20493. By this method it is possible to determine sulphur quickly and accurately, and it is preferable to the gravimetric method if nitrogen and halogens are absent.

In Presence of Nitrogen, Chlorine and Bromine.[7] The absorbing solution and the washings from the bead-tube are collected in a platinum or clear, transparent quartz dish. One drop of phenolphthalein is added, and 0·02 *N* sodium hydroxide solution is run in from a burette until a clear red colour is obtained, and the reading is taken. The dish is then placed on an open water-bath for 20–30 mins., and 0·02 *N* sulphuric acid equivalent to the alkali previously taken is run in from a burette.

The solution is stirred well and evaporated to dryness on the water-bath ; the residue is dissolved in a little water and again evaporated completely to dryness. Water is then again run on to the crust of salt, and the residue is allowed to stand for 45 mins. on the boiling water-bath. Evaporation three times is essential to drive off the volatile acids, and to convert the sulphuric acid originally present into the sodium bisulphate to be titrated (see equations below).

For the titration, the residue is dissolved in 5–8 ml. of hot water, methyl red is added, and the solution is titrated with 0·02 *N* sodium hydroxide solution until the first appearance of the yellow colour ; the dish is then placed for some minutes on the water-bath and titrated while hot to the canary-yellow colour.

Because in practice it is never certain that all volatile acids have been completely removed before the titration, a blank titration must be

made. If this is found to be negligible, it may be omitted in further analyses of the same or similar substances. For this purpose a volume of 0·02 *N* sulphuric acid equivalent to the total alkali used for neutralisation and for the bisulphate titration is run into the dish, which now contains sodium sulphate. The procedure described above is followed, and the residue is titrated as above with 0·02 *N* alkali. If all the volatile acids have been removed, all the sulphuric acid added in the control test will be titrated back. Now, if 1 gm.-mol. of sulphuric acid and 2 gm.-mols. of hydrochloric acid are present after the combustion, 4 gm.-mols. of sodium hydroxide are required for neutralisation :

$$H_2SO_4 + 2HCl + 4NaOH = Na_2SO_4 + 2NaCl + 4H_2O.$$

On evaporation with 2 gm.-mols of acid :

$$Na_2SO_4 + 2NaCl + 2H_2SO_4 = 2NaHSO_4 + Na_2SO_4 + 2HCl.$$

$\therefore$ 1 ml. 0·02 *N* NaOH = 0·3206 mgm. sulphur.

Factor = 0·3206. Log (factor) = $\bar{1}$·50596.

Log (%S) = log (ml. 0·02 *N* NaOH) + log (factor) + 2 − log (mgm. sample).

Substances containing iodine give low values ; 3–4 hrs. are necessary for an analysis, which is accurate to within ± 0·2%. The method is strongly recommended for students, but for the general work of the microchemical laboratory where one has to reckon with a variety of substances, the gravimetric process is given preference.

Direct Titration. Within recent years much work has been carried out on indicators for the direct titration of sulphates with barium chloride. Hallett and Kuipers [8] have obtained good results with tetrahydroxy quinone, and have shown that despite a rather indistinct end-point any chosen stage of the colour-change is reproducible with sufficient precision for routine work. The rhodizonic acid and barium chromate indicators now well-known in this connexion have also been used, but with less success.[11] Ingram [10] describes a method in which an excess of barium chloride is added followed, after evaporation, by back-titration with mercuric oxycyanide. Modifications in the combustion apparatus ensure the elimination of errors due to the formation of sulphur trioxide.

The well-known benzidine method has also been adapted by Marsden and Pollard [9] to the same end, and so long as certain salts are absent (in particular, chlorides, nitrates and phosphates) and the working conditions are carefully adjusted, the method is reliable ; however, these provisos eliminate it for many purposes. The sulphate solution, adjusted to *p*H 3, is precipitated by adding to 4 ml. of solution 1 ml. of a fresh, filtered 0·8% solution of benzidine chloride. The mixture is immersed in crushed ice for 10 mins. and centrifuged, and the precipitate is washed, by decantation, with 5 ml. of 80% alcohol. The washing is repeated, the last traces of alcohol are removed by immersion of the container in hot water, and a solution of the residue in 5 ml. of 0·5% potassium hydroxide solution is diluted to 25 ml., and mixed with 1 ml. of concentrated sulphuric acid. The hot mixture is

titrated with 0·05 N potassium permanganate solution, and when the apparent end-point is reached, an excess equal to 25% of the volume already used is added, followed by an extra 1·0 ml., and after 10 mins. on the water-bath, by 2·0 ml. of 0·05 N sodium oxalate solution. The precipitated manganese dissolves, and the titration is completed with the permanganate solution

Then, (total titration $-$ 2) $\times$ 0·394 = mgm. of S.

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DETERMINATION OF PHOSPHORUS

Those of the many methods for the determination of small amounts of phosphorus which have proved best for the analysis of crystalline organic compounds are described below.

The organic substance is destroyed, either by fusion with sodium carbonate and potassium nitrate, or by boiling with a mixture of nitric and sulphuric acids, which oxidise the phosphorus to phosphoric acid. The acid is precipitated quantitatively with the Lorenz [1] ammonium molybdate reagent, and weighed as ammonium phosphomolybdate or determined volumetrically by dissolving the precipitate in an excess of standard sodium hydroxide solution, boiling off the ammonia, and titrating back the excess of alkali. A precision of $\pm$ 0·1% is easily attained, because the precipitate weighed is 63 times as heavy as the phosphorus which it contains ; or 30 times as heavy as the phosphorus pentoxide. The conditions of preparation of the reagents and of precipitation must be observed most strictly, because the factor (see Lorenz,[1] Lieb and Wintersteiner,[2] and Kuhn [3]) has been ascertained empirically, and holds only for the specified conditions. The gravimetric and volumetric methods are equally accurate, but the latter is the less rapid because of the rinsing and evaporation processes it involves. The Carius method has also been used, but it often gives low results owing to absorption of phosphoric acid by the glass.[4]

Reagents

Sulphate-Molybdate Reagent. Fifty gm. of ammonium sulphate are dissolved in 500 ml. of nitric acid (d, 1·36) in a graduated litre flask ; 150 gm. of powdered ammonium molybdate are dissolved in 400 ml. of boiling water in a beaker, and cooled. The latter solution is poured

slowly, with stirring, in a thin stream into the former. The mixture is then diluted to 1 litre, allowed to stand for 3 days, and filtered through an ordinary filter into a brown bottle which is kept well stoppered.

Dilute Nitric Acid. (1 : 1).

Nitric Acid Containing Sulphuric Acid. Thirty ml. of sulphuric acid (*d*, 1·84) are poured into 1 litre of nitric acid (*d*, 1·19–1·21), which is obtained by mixing 420 ml. of nitric acid (*d*, 1·40) with 580 ml. of water.

A 2% Aqueous Solution of Pure Ammonium Nitrate. This should be made weakly acidic with nitric acid.

Alcohol, 95–96% by volume.

Ether. This should be free from alcohol and water. At room temperature, 150 ml. of ether should dissolve 1 ml. of water to give a clear solution.

Acetone. This must be analytical grade, and free from aldehydes.

Soda-Nitrate Mixture. Equal weights of the purest, finely-powdered sodium carbonate and potassium nitrate mixed.

For wet oxidation **sulphuric acid** (*d*, 1·84), **nitric acid** (*d*, 1·4), and acid-free **hydrogen peroxide** (perhydrol grade) are required.

Procedure

Dry Decomposition. The substance (2–5 mgm.) is weighed into a platinum boat, well mixed with 5–6 times its weight of the mixture of sodium carbonate and sodium nitrate, by means of a thin platinum wire, which is left in the boat. The boat is placed in a combustion tube (length, 15 cm.) which has been carefully cleaned with sulphuric-chromic acid. The tube is bent at right angles at one end, which is drawn out to a capillary. The other end is closed by a rubber stopper; oxygen is passed through a narrow capillary in this at 3–4 ml. per min. After the oxygen is introduced, the tube is first heated in front of the boat, and the flame is gradually advanced up to the boat. Immediately the main reaction is over, the boat is heated from beneath with the full, non-luminous flame, and then allowed to cool in the current of oxygen.

The boat is then placed in a test-tube, the fused mass dissolved in about 5 ml. of boiling dilute nitric acid, and filtered through a moistened hard filter into a 100-ml. test-tube (cleaned with sulphuric-chromic acid). If particles of the melt have spirted into the combustion tube, this also must be rinsed out with the hot dilute nitric acid. When the material has dissolved, the clear solution is run out through the capillary while rotating the tube.

To the cooled filtrate are added 2 ml. of nitric acid containing sulphuric acid, and sufficient water (if necessary), to make a total volume of 15 ml.

Wet Decomposition. The substance is weighed into a small, dry Kjeldahl flask (cleaned with sulphuric-chromic acid) by means of a nitrogen weighing tube (p. 73). To this are added 0·5 ml. of concentrated

sulphuric acid and 4–5 drops of the concentrated nitric acid. The mixture is then heated over a small flame until sulphur trioxide fumes are evolved, and the process from the addition of the nitric acid is repeated twice more. If, after cooling, the solution is still not clear, 4–5 drops of perhydrol are added and the material is again heated until the fumes appear. This is repeated until the solution is quite clear, when the flask is rinsed into a wide-necked test-tube.

Precipitation. The test-tube containing the solution obtained by either method is placed in a litre beaker on the boiling water-bath while the molybdate reagent is filtered. The test-tube is removed from the water-bath, 15 ml. of reagent are run into it from a pipette (without touching the side), and after 2–3 mins. the tube is shaken well and then left to stand for at least 6 hrs. to precipitate the ammonium phosphomolybdate completely. According to Kuhn,[3] the quantitative separation of a precipitate equivalent to less than 0·5 mgm. of phosphorus takes 6–18 hrs., and for less than 0·05 mgm., up to 36 hrs. It should be noted that after precipitation is complete the test-tube must not be heated again on the water-bath, or precipitation of free molybdic acid will occur.

During the separation of the precipitate, a filter-tube (p. 88) is prepared ; a tube which has already been used is cleaned by dissolving out the ammonium phosphomolybdate with ammonia. It is washed, in either case, with water, hot dilute nitric acid, and distilled water successively, and the water is finally removed by filling up twice with alcohol, ether, or acetone, and emptying. The filter-tube is then wiped with a moist flannel and dry chamois leather, and left for at least 30 mins. in an empty desiccator (containing no drying agent) which is evacuated with the water pump. The disappearance of all smell of ether or acetone indicates that the tube is ready for weighing.

The filter-tube is removed from the desiccator immediately before use and the time noted ; 5 mins. should then elapse before it is weighed. Later, the weighing of the precipitate should be carried out after exactly the same interval.

Filtration proceeds as described on p. 93. After the supernatant liquid has been syphoned off, the precipitate is washed thoroughly with the 2% ammonium nitrate solution and transferred to the filter. To remove the last traces of precipitate the walls of the test-tube are rinsed well with ammonium nitrate solution and 95% alcohol alternately, and the tube is finally filled twice with alcohol and ether or acetone to remove the water. After wiping with the flannel and chamois leather, the tube is weighed under the same conditions as before. Because the weight of the ammonium phosphomolybdate is much greater than that of the phosphorus contained in it, weighing to $\pm$ 0·01 mgm. is sufficiently accurate.

Calculation

The weight of the precipitate multiplied by the empirically-

determined factor, $F = 0{\cdot}01454$, gives the weight of phosphorus. $\log F = \bar{2}{\cdot}16249$

$$\log (\% \text{ P}) = \log (\text{wt. precipitate}) + \log F + 2 - \log (\text{wt. sample}).$$

Example: Diphthaloyl-phosphoric acid ester, 5·925 mgm. yielded 36·08 mgm. of ammonium phosphomolybdate.

Theoretical for $C_{20}H_{13}PO_4$ (mol. wt., 348·22): P = 8·91%.
Found: P = 8·85%.

Notes

The Volumetric Method was developed from Blair's alkalimetric determination of phosphoric acid in iron and steel. In the initial stages the method is identical with the gravimetric determination, except that the precipitation is carried out in a 100-ml. beaker. After the mixture has stood overnight, the supernatant liquid is poured through a hard filter and the precipitate is washed with a little ice-cold 50% alcohol. The particles on the filter are carefully rinsed back into the beaker.

The precipitate is dissolved in 0·1 *N* alkali, using twice as much as will just dissolve it, and the ammonia is boiled off for at least 30 mins.; the final volume is about 10 ml. To the alkaline solution, 2–3 drops of 1% phenolphthalein (or thymophthalein) solution are added, about 3–5 ml. in excess of 0·1 *N* acid are run in, the solution is boiled for 10–15 secs., cooled, and titrated back with 0·1 *N* sodium hydroxide solution.

The phosphorus present is calculated according to Iversen's equation; 1 atom P = 28 equivalents of alkali:

$$(NH_4)_3PO_4.12MoO_3.2HNO_3 + 28NaOH =$$
$$12Na_2MoO_4 + 2NaNO_3 + Na_2HPO_4 + 16H_2O + 3NH_3.$$

The 0·1*N* alkali used, multiplied by the factor $F_1 = 0{\cdot}1108$, gives the amount of phosphorus in mgm.; $\log F_1 = \bar{1}{\cdot}04455$.

Embden's Method. The phosphoric acid is precipitated as strychnine phosphomolybdate. The ratio of phosphorus to strychnine phosphomolybdate is 1 : 98. The precipitation can be carried out in the cold, and therefore it is practically quantitative in a short time, and filtration can proceed after only 30 mins.; this is very convenient for physiological samples, as the danger of liberation of free phosphoric acid from organic phosphates is minimised.

If the organic substance also contains arsenic, then a mixture of phosphoric and arsenic acids is obtained after the decomposition, and the latter must be removed before precipitation. Kuhn [3] has worked out a micro-separation of phosphoric acid and arsenic acid. The pentavalent arsenic is reduced with hydrazine in hydrochloric acid, and the trichloride is distilled off in a slow stream of hydrogen chloride. The phosphorus is evaporated with nitric or hydrochloric acid according as it is to be determined gravimetrically or nephelometrically (p. 222), respectively.

Other micro-methods for the determination of phosphorus are dealt with by Feigl, Strebinger and Barrenscheen.[7]

Simultaneous Determination of Barium and Phosphorus. In barium salts of organic esters of phosphoric acid, barium cannot be determined by evaporation with sulphuric acid, and accordingly the following method is recommended (error, 0·2%). About 2–5 mgm. of the substance are weighed from the long-handled weighing tube (p. 73) into a carefully cleaned and dried small Kjeldahl flask ; 0·5 ml. of concentrated sulphuric acid (*d*, 1·84) is added from a graduated pipette, and the substance is decomposed over the smallest possible Bunsen flame. The flask is heated until fumes of sulphur trioxide appear, it is allowed to cool, and 5–8 drops of perhydrol (p. 100) are added from a dropping pipette ; the flask reheated until the perhydrol is destroyed and sulphur trioxide fumes escape from the neck of the flask. This process is repeated until the solution is quite clear, which is usually after three additions of perhydrol, and the barium sulphate is then in solution in the anhydrous sulphuric acid.

The flask is then allowed to cool and the walls are rinsed down carefully, with shaking, with a fine stream of 3 ml. of distilled water, when the barium sulphate is precipitated immediately. The contents of the flask are then rinsed quantitatively, with a further 3 ml. of water, into a glass crystallising dish which has been cleaned with sulphuric-chromic acid, and this is covered with a clock-glass.

Meanwhile, the Neubauer platinum crucible is cleaned, ignited, and weighed ready for the filtration of the barium sulphate (p. 105). For preference, a wide-necked filter-flask containing a 25-ml. test-tube for the collection of the filtrate is used. The barium sulphate can be rinsed into the crucible without difficulty with 2–3 ml. of water. Finally, the crucible is raised somewhat out of its collar, and its base and the collar are rinsed inside with 1–2 ml. of water. With its lower cap and lid, it is placed on a large platinum lid and ignited and weighed (p. 105).

The test-tube containing 10–12 ml. of solution and washings is taken out of the flask with forceps, and ammonium phosphomolybdate is precipitated by the addition of 2 ml. of nitric acid containing sulphur (p. 110).

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DETERMINATION OF ARSENIC

The general procedure is to destroy the organic matter by wet oxidation with sulphuric acid and nitric acid, or preferably hydrogen

peroxide,[1] and to determine the arsenic in the resulting solution by one of the following methods :

1. *Gravimetrically*, preferably as magnesium pyroarsenate (Lieb and Wintersteiner [2]).

2. *Volumetrically*, preferably by titration with 0·01 *N* thiosulphate solution after addition of potassium iodide ; this method is preferable to the gravimetric method for many purposes. Kolthoff and Amdur [3] suggest, as an alternative, reducing the arsenic to the elemental state, dissolving it in an excess of standard ceric sulphate solution, and back-titrating with arsenious acid ; errors of only 0·5% for 1 mgm. and 2% for 0·1 mgm. of arsenic are claimed. A variation on this same method is the use of a bromide-bromate solution as solvent for the reduced arsenic.[4]

3. *The Marsh Test*, suitably modified if necessary. This test is, of course, essentially a micro-method in its usual form, but it is preferable to dissolve the arsenic mirror and titrate it.

4. *Electrodeposition* (see p. 123).

Many references to micro-methods for arsenic are given (with summaries of the methods) by Heller,[5] by How,[6] and by Jacobs and Nagler.[7]

Gravimetric Method

Procedure

(*a*) *Decomposition in the Pressure-Tube.* With the aid of the weighing tube with the long handle (p. 73), 5–10 mgm. of the sample are weighed into a small pressure-tube (cleaned with sulphuric-chromic acid) ; about 0·5–1 ml. of concentrated nitric acid is added from a pipette, while rotating the tube. The tube is sealed (p. 97) and heated for several hours in the small tube-furnace at 250°–300° C., according to the resistance of the substance to decomposition. After opening the tube (p. 98), the contents are rinsed quantitatively with distilled water into a 30-ml. glass dish.

(*b*) *Decomposition in a Micro-Kjeldahl Flask.* To the sample on the bottom of the dry flask are added 5 drops of 30% sulphuric acid and 0·5 ml. of perhydrol. It is heated (see p. 82), if necessary with a fresh addition of perhydrol. When all the material is decomposed and the solution is clear, the solution is rinsed quantitatively into the glass dish.

Precipitation and Weighing. The solution is evaporated to dryness on a water-bath and the residue dissolved in 3–4 ml. of 2·0 *N* ammonia, and 1 ml. of magnesia mixture (prepared by dissolving 5·5 gm. of crystallised magnesium chloride and 10·5 gm. of ammonium chloride in 100 ml. of water) is added from a pipette. As the precipitate which separates is amorphous it must be allowed to stand for at least 6 hrs., after which it is crystalline and is easily filtered in a micro-Neubauer crucible (as described on pp. 89 and 103), and washed with 2·0 *N* ammonia. The last traces are transferred to the crucible by the alternate use of 2·0 *N* ammonia and alcohol. The crucible is then

removed from the rubber collar, closed with the lid and lower cap, and strongly ignited on a large platinum lid. The magnesium pyroarsenate thus obtained still occludes magnesium salts, and must therefore be washed thoroughly with very weak ammonia water. After strong ignition the crucible is brought to the balance on the copper block of the micro-desiccator, and it may be weighed after 10 mins. Further washing usually results in a loss of weight of only 0·01 mgm. The method gives results which differ from theoretical by 0·2% at most, and are usually slightly low.

Calculation

og (% As) = log (wt. $Mg_2As_2O_7$) + log 0·4827 + 2 − log (wt. sample)

$$\text{Factor} = \frac{\text{As}}{\text{Mg}_2\text{As}_2\text{O}_7} = 0{\cdot}4827$$

$$\log 0{\cdot}4827 = \bar{1}{\cdot}68368.$$

Volumetric Method [8]

Reagents

Sulphuric Acid. 30%.

Nitric Acid. Sp. gr., 1·4.

Hydrogen Peroxide. Acid-free perhydrol grade (p. 100).

Hydrochloric Acid. About 25 ml. of concentrated acid are boiled gently for exactly 2 mins. in a 100-ml. conical flask, to remove free chlorine. A glass stopper is then immediately inserted, and the flask is cooled under the tap.

Potassium Iodide. A colourless 4% solution is used.

Thiosulphate Solution. 0·01 *N*. To 25 ml. of matured 0·1 *N* thiosulphate solution in a 250-ml. graduated flask, are added 2·5 ml. of amyl alcohol (A.R. grade), and boiled water up to the mark. The solution is stored in a brown bottle, and the factor is determined (p. 173) after 2 days with 0·02 *N* chromic acid (using 3 ml. of concentrated hydrochloric acid instead of sulphuric acid for acidification) or with potassium iodate The latter (2·5–3·0 mgm.) is dissolved in 5 ml. of boiled water, and 3 ml. of the above hydrochloric acid and 2 ml. of the 4% potassium iodide solution are added. After 2 mins. the iodine liberated is titrated. Towards the end of the titration the liquid is diluted to 20 ml., starch solution is added, and the titration completed.

Starch Solution. See p. 173.

Procedure

The sample (7–12 mgm.) is weighed into a small dry Kjeldahl flask (p. 79); particles adhering to the walls are rinsed down with 1 ml. of 30% sulphuric acid. After adding 5 drops of concentrated nitric acid the flask is heated over a small flame. Immediately white sulphur trioxide fumes appear add 5 drops more of nitric acid and

re-heat. Finally, 5 drops of perhydrol are added. The solution should be, and remain, clear on cooling; otherwise, repeat the additions. After cooling, in order to remove the hydrogen peroxide completely, and more particularly to destroy excess of oxypersulphuric acid, the liquid is evaporated three times with 1 ml. of water each time, until SO_3 fumes appear and strong sulphuric acid condenses on the walls of the flask. After again adding 1 ml. of water the liquid is boiled for 1 min. and its contents are poured into a wide-necked bottle or 100-ml. conical flask, 5 ml. of pure, boiled hydrochloric acid being used for rinsing.

For the titration, 2 ml. of potassium iodide solution are added and the mixture is allowed to stand for 10 mins. in the flask, which is closed by a glass stopper. The liberated iodine is titrated with 0·01 *N* thiosulphate solution from a 10-ml. micro-burette with a glass cock. When the solution is faintly yellow, the liquid is diluted to a 20-ml. mark with boiled water, 4 drops of starch solution are added and the titration completed. A faintly reddish tint is the end point; the blue colour reappears only after 5–10 mins.

The values obtained for substances containing bromine, or especially iodine, are high. To remove iodic acid, 0·3 ml. of the potassium iodide solution and 1 ml. of water are added to the flask after the oxidation and destruction of the oxypersulphuric acid. The liquid is heated until all the iodine is volatilised, and then again with perhydrol until SO_3 fumes appear, to oxidise the partly reduced arsenic acid. Finally, it is evaporated twice, with 1 ml. of water, to destroy the perhydrol and oxypersulphuric acid.

Blank Test. This is necessary despite the use of boiled solutions, as iodine is usually liberated by the action of air on the reagents. To 1 ml. of 30% sulphuric acid in a test-tube is added 1 ml. of water, the mixture is boiled and the contents rinsed into the titration vessel with 5 ml. of boiled hydrochloric acid; 2 ml. of the 4% potassium iodide solution are added, the liquid is allowed to stand for 10 mins. in the stoppered flask, and then diluted to the 20-ml. mark. Starch is added, and the solution is titrated with the 0·01 *N* thiosulphate solution. The volume required varies from 0·04–0·08 ml.

Calculation

$$2HI + H_3AsO_4 = H_2O + I_2 + H_3AsO_3.$$

Therefore, 1 atom of arsenic, corresponds with 2 atoms of iodine, and 1 ml. of 0·01 *N* thiosulphate with 0·3748 mgm. of arsenic; log 0·3748 = $\bar{1}$·57380.

$$\log (\%\ As) = \log (ml.\ 0{\cdot}01\ N\ Na_2S_2O_3) + \log 0{\cdot}3748 + 2 - \log (wt.\ sample).$$

Example. 9·417 mgm. of phenyl-arsenious acid required 9·36 ml. of 0·01 *N* $Na_2S_2O_3$.

$$\text{Percentage of arsenic} \begin{cases} \text{Theory, } 37{\cdot}18. \\ \text{Found, } 37{\cdot}36. \end{cases}$$

Micro-Marsh Method [9]

Procedure

To the sample (2–3 gm.) in a conical flask is added 1 ml. of 50% sodium chlorate solution, the flask is closed with a stopper carrying two tap-funnels and an air-condenser, and 4 ml. of fuming hydrochloric acid (sp. gr., 1·19) are added from one of the former. The flask is heated on a water-bath, with shaking, and 1- and 2-ml. portions of acid are added successively. The frothing mixture is removed from the bath, and 1 ml. each of the acid and chlorate solution are added simultaneously, using both funnels. After further heating decomposition should be complete, and the cool solution is diluted with 30% hydrochloric acid, and warmed to remove the chlorine.

The reduction to an arsenic mirror is carried out with spongy tin so as to eliminate interference by antimony; this is prepared by immersing zinc in a dilute solution of stannous chloride in hydrochloric acid, filtering off the precipitated tin, washing it well and heating it in boiling 50% hydrochloric acid for 1–2 hrs. to remove any arsenic. To the reaction flask are added 20 gm. of the tin, 6·5 ml. of fuming hydrochloric acid and sufficient water to cover the tin. The tube is closed by a stopper carrying an inlet tube connected (via wash bottles containing potassium permanganate solution and strong sulphuric acid) with a hydrogen cylinder; and a 10-ml. pipette bent at right-angles. The other end of the pipette is connected through a quartz spiral [10] (internal diameter, 0·2 mm.) to a tube dipping into water. Lengths of woollen thread are wound around the pipette and the portion of the spiral at which the mirror is to be formed, and are kept moistened continuously with cold water.

The hydrogen is turned on, and after 10–15 mins. the spiral is heated with a small fish-tail burner. The flask is then gently heated so that boiling starts in 30–40 mins., when the hydrogen is cut off, and after boiling for a further 10 mins. both flames are removed, and the apparatus is allowed to cool in a current of hydrogen.

The arsenic mirror may be matched in the usual way, or, preferably, it is dissolved in 0·25 ml. of an iodine-chloride reagent prepared by pouring a solution of 1·56 gm. of potassium iodide and 1 gm. of potassium iodate in 50 ml. of water into 50 ml. of concentrated hydrochloric acid, and adding dilute potassium iodate solution until a drop of carbon tetrachloride solution added to the mixture develops no colour. To the solution are added 0·3 ml. of 10% potassium cyanide solution and 3 drops of carbon tetrachloride, and 15 mins. after vigorously shaking, the mixture is titrated with 0·001 *M* potassium iodate solution, until the carbon tetrachloride layer is colourless. A blank test must be carried out on the reagents.

The working time required is about 3 hrs., and 0·3–0·5 gm. of arsenic can be determined with a maximum error of $\pm$ 3·5%.

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DETERMINATION OF METALS IN ORGANIC COMPOUNDS

Such determinations are simple in that they do not differ in principle from the corresponding macro-methods. On account of the low conversion factor, however, great care is necessary, especially when only small samples are available. If the metals are not to be determined simultaneously with the carbon and hydrogens (p. 60) the sample is ignited in a platinum or porcelain crucible or in a small boat (Pregl). Thus :

(*a*) Metal oxide residues are weighed after direct ignition.

(*b*) Metals are weighed as sulphates, after evaporation with sulphuric acid.

Apparatus

The Crucible with lid is about 15 mm. high, and has upper and lower diameters of 12 and 10 mm., respectively. The residue remaining from previous determinations is removed by cleaning with nitric acid, sulphuric acid, fused potassium sulphate and water successively. The platinum crucible is ignited on a platinum lid (diameter, 30–40 mm.) which protects it from direct contact with the gases of the flame ; this lid rests on a silica triangle. The porcelain crucible heated in a protecting porcelain crucible over an open flame, but if the ignition time is very short, the protecting crucible is unnecessary. The boats are identical with those used for the carbon and hydrogen determinations (see p. 53).

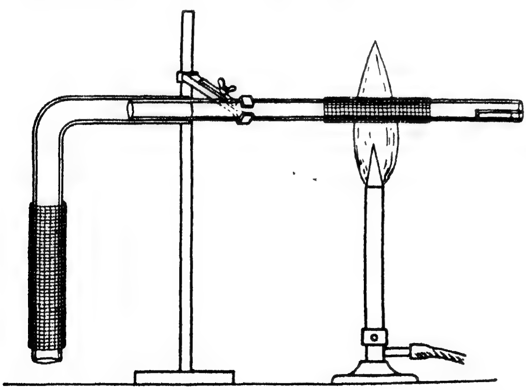

FIG. 61. Micro-muffle for determination of metals. One-third actual size.

The Micro-Muffle (Fig. 61) consists of a Supremax-type glass tube (length, 200 mm. ; external diameter, 10 mm.), *e.g.*, an old combustion tube, clamped horizontally at such a height that it can be heated by

the hottest part of a Bunsen flame. The shorter limb of hard glass tubing (inner diameter, 12–14 mm.), bent at right angles, is placed over one end of the muffle tube with a layer of asbestos paper between the tubes. The limbs are 50 and 150 mm. long, and the latter is wrapped in a double layer of wire gauze, 80 mm. long, which is heated by the flame of an inclined Bunsen burner, so that an ascending air-current is induced within the tube. This attains a higher velocity in the horizontal tube in consequence of the reduction in diameter. A roll of wire gauze (length, 50 mm.) over the horizontal tube enables this current of air to be uniformly heated with a second burner.

Procedure

Crucible Method. The clean crucible is ignited for 5 mins. and weighed to within $\pm$ 0·001 mgm. after 5 and 20 mins. for platinum and porcelain, respectively. The sample (2–5 mgm.) is weighed into the crucible with a micro-spatula, which is carefully brushed with a marten-hair brush. For hygroscopic substances, a stoppered weighing tube (p. 19) is used. Oils are placed on the base of the crucible by means of a glass thread. The lid is replaced, and the crucible is transferred to the large platinum lid or the protecting crucible.

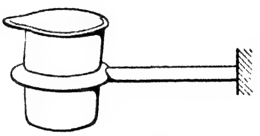

FIG. 62. Platinum crucible in ring, for determination of noble metals. Actual size.

The non-luminous flame of a Bunsen burner is directed carefully on to the lid of the micro-crucible. Immediately the substance has charred, the base of the crucible is heated carefully with a higher flame. After about 5 mins. the flame is turned out, the lid removed, and, if carbon is still to be seen in the crucible, 1 drop of nitric acid (sp. gr., 1·4) is added from a glass thread. With very resistant substances, the addition of nitric acid must be repeated frequently.

The crucible is now placed on the copper block and again weighed. If the material is difficult to ash, the ignition with nitric acid and weighing are repeated until the weight is constant. Even compounds containing gold and platinum may usually be analysed accurately in this way.

The crucible and contents are now placed in a platinum ring (Fig. 62), which is heated carefully over a micro-burner. The hydrochloric acid and the products of combustion from the organic material escape. The micro-burner is then removed and the crucible heated, with a just non-luminous flame, for 3 secs. at a dull red-heat. If any carbon still remains in the crucible, ignition is repeated. The results should not be more than 0·2% less than the theoretical values.

Boat Method. To the weighed sample in a platinum or porcelain boat is added 1 drop of dilute sulphuric acid (1 : 5), which is allowed to fall from a vertical capillary drawn out to a fine point. The boat is then inserted, by means of platinum-tipped forceps, into the open end of the horizontal tube (Fig. 61), and the roll of wire gauze is placed

about 30 mm. in front of the boat and heated at the middle with a Bunsen burner. The roll and burner are gradually brought nearer to the boat, but if this is done too rapidly the contents of the boat may creep over the edge or froth violently. When the burner is under the boat faint fumes of sulphur trioxide appear at the mouth of the tube, and the wire roll is then removed and the boat strongly ignited for 1 min. to convert the acid sulphate into the normal salt. If particles of carbon are still visible, the boat is held by the platinum-tipped forceps and ignited for about 2 secs. in a roaring flame, placed on the copper block and weighed after 5 and 10 mins. for platinum and porcelain, respectively. Meixner and Kröcker [1] found that when using the micro-muffle, trouble was experienced with many substances owing to swelling ; this is avoided in the crucible method.

Determination as Sulphate. To the weighed sample in a crucible is added 1 drop of dilute sulphuric acid (1 : 5) from a pointed capillary tube about 200 mm. long and 1–2 mm. in diameter. After replacing the lid, the material is heated momentarily from above for periods of 1–2 secs., with a micro-burner, so that each time slight fumes of sulphur trioxide escape. When the sulphuric acid has evaporated, the crucible is heated from below for 3 mins. with a stronger flame, to convert all the bisulphate into sulphate. If any carbon still remains, 1 drop of nitric acid is added and the residue is ignited again ; this is repeated until all carbon has been destroyed, and the residue is heated finally with sulphuric acid.

Calculation

(*a*) Found : metal.

$$\log (\%\ \text{metal}) = \log (\text{wt. residue}) + 2 - \log (\text{wt. sample}).$$

(*b*) Found : metallic oxide or sulphate.

$$\log (\%\ \text{metal}) = \log (\text{wt. residue}) + \log F + 2 - \log (\text{wt. sample}).$$

For factor F, see p. 229.

Example : 4·584 mgm. trimethyl heptadecabetaine chloro-platinate yielded 0·845 mgm. of platinum.

Molecular weight, $2(C_{20}H_{41}NO_2),H_2PtCl_6 = 1064{\cdot}7$.

$$\text{Platinum}\begin{cases}\text{Theory}: & 18{\cdot}34\%.\\ \text{Found}: & 18{\cdot}43\%.\end{cases}$$

Notes and Other Methods. Direct ignition, if necessary with nitric acid, enables the following to be determined :

In *Platinum Crucibles* : Iron as Fe_2O_3 ; aluminium as Al_2O_3 ; copper as CuO ; tin as SnO_2 ; silicon as SiO_2 ; magnesium as MgO.

In *Porcelain Crucibles* : Chromium as Cr_2O_3 ; silver, gold, and platinum as metal.

If salts of noble metals have frequently to be analysed it is advisable to keep a special crucible for them. Platinum crucibles retain their weights better than the porcelain crucibles, and enable the determination to be made much more rapidly. When about 500 mgm. have

accumulated, the silver and gold are removed electrolytically, using the crucible as anode.

Metals which may be determined as sulphates are, sodium, potassium, magnesium, calcium, strontium, barium, cadmium, manganese, and lead. With organic salts of lead, addition of nitric acid is essential, because the crucible is damaged by elementary lead. Lithium compounds are heated with sulphuric acid in the micro-muffle ; the sulphate is hygroscopic, and must be weighed with due precautions.

Meyer and Hoehne [2] found that results having an error of $\pm$ 1% are obtained with iron, chromium and vanadium organic compounds by burning 5–30 mgm. of sample in oxygen for 30 mins. and weighing the sample as oxide ; if desired, reduction with hydrogen may be used to obtain the metal (*e.g.*, with cobalt or nickel). Calcium, barium, sodium, rubidium, caesium and potassium, however, are determined by the sulphate method (Roth [3]).

For further information see the papers by Heller,[4] dealing with the determinations of 11 common metals ; and by Hallett,[5] who gives numerous appropriate references to, and brief descriptions of recent methods used for the determination of individual metals in the residue left after the treatment described above.

Finally, attention may be drawn to the following section, which deals specifically with electrodeposition methods for the determination of metals.

Mercury [1]

A 12-mm. layer of calcium oxide is placed in an ordinary micro-combustion tube (p. 36), at about 5 cm. in front of the neck ; it is held in place by asbestos plugs. The tube so filled is ignited on a stand (p. 34) in a current of air, as in the halogen determination (p. 91). After water from any calcium hydroxide present has been driven off, the tube is further heated to redness with a tube burner. Meanwhile, from 3–8 mgm. of the substance to be analysed are weighed into a

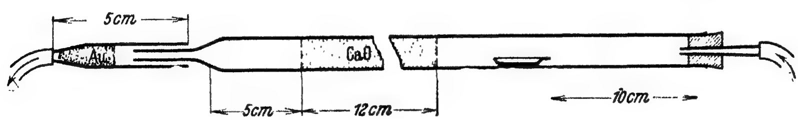

FIG. 63. Micro-determination of mercury.

porcelain boat, and this is pushed up to about 4 cm. in front of the layer of calcium oxide. A small tube filled with very fine gold wire and weighed accurately to 0·001 mgm. is placed over the neck of the combustion tube (Fig. 63). The constricted end of the small tube is connected with the Mariotte flask by rubber tubing, so that air is aspirated through this end somewhat faster than it enters the combustion tube. Hence diluting air can be drawn in at the place at which the tube passes over the combustion tube, without loss of air from the latter.

The substance is burnt in the usual way by moving a burner forward ; the products of combustion thus pass into the ignited layer of lime. The mercury vapour passes through the filling and first condenses at the cold end of the tube, in front of the neck ; chlorine and sulphur dioxide are retained by the lime. By means of a small flame, the mercury is driven carefully into the tube containing gold, the end of which is cooled with a small wet chamois leather. After 1 litre of air has been aspirated through, the tube of gold is taken off, wiped, and allowed to stand for 30 mins. in a desiccator containing calcium chloride at atmospheric pressure.

Simultaneous Determination of Nitrogen and Mercury.[6] The method is based on the fact that, in the Pregl-Dumas method for the determination of nitrogen, the mercury collects quantitatively in the cold projecting part of the combustion tube. After the removal of the nitrometer, the mercury is driven into the neck of a glass tube containing gold, as in the above method for mercury. This tube (length, 45 mm. ; external diameter, 10 mm.) is blown out at one end, while at the other it is constricted and passes into a capillary (length, 35 mm. ; external diameter, 3 mm.). The tube filling is a layer of tightly compressed fine gold wire, 15 mm. long.

Since carbon dioxide generated from marble and hydrochloric acid contains hydrochloric acid (which may produce volatile compounds of copper), Hernler used Schöller's apparatus [7] for the production of this gas from potassium-sodium carbonate (which has been fused *in vacuo*) and dilute sulphuric acid. For the expulsion of the mercury, which is carried out in a slow stream of carbon dioxide, fine grooves are made in the stopcock of the gas delivery tube of the carbon dioxide apparatus. The sample (3–7 mgm.) is mixed with copper oxide, and placed in the combustion tube as previously described. The tube is placed on the combustion stand so that 80 mm. at the neck end project, and it is cooled with a moist flannel just before the junction with the neck to prevent the mercury entering the neck prematurely.

Combustion is carried out exactly as described on p. 74, and if at the conclusion tiny gas bubbles again rise in the nitrometer, the velocity of the gas is adjusted at the generator (by means of the grooved stopcock) to 2 bubbles per sec. The stopcock H_2 (Fig. 42) is also opened. After removing the nitrometer the moist flannel is taken away, the neck of the combustion tube is wiped with a clean towel, a short roll of wire gauze is placed over the end of the tube, and the tube containing gold (which has been weighed accurately to within 0·001 mgm.), is pushed over the neck of the combustion tube until this touches the gold. The capillary end of the tube of gold is connected with the Mariotte flask, by means of which air is aspirated through at twice the rate of the carbon dioxide ; a moistened flannel is placed around the constriction in the tube.

Now any mercury which has distilled back is driven up to the tube burner by the moveable burner, the flame of which is as small as

possible ; any residues which are difficult to volatilise may be neglected. Air is then aspirated through by means of the Mariotte flask rather more rapidly, and the mercury is driven over into the gold by heating with a very small flame and moving the gauze roll slowly forward.

When the burner has reached the tube of gold, it is kept there and the mercury is driven over into the tube with a micro-burner. To remove the last traces, the tube is heated once more with the burner and finally with the micro-burner. The flame should be too small to produce a visible glow on the gauze. Suction with the Mariotte flask is then increased, and after about 1,500 ml. of air have been drawn through, the tube of gold is wiped first with a moist flannel, then with chamois leather, and placed on the balance pan and weighed after 20–30 mins.

The error of the method is $\pm$ 0·5%. The mercury is removed from the gold before the next determination by warming gently in the current of air.

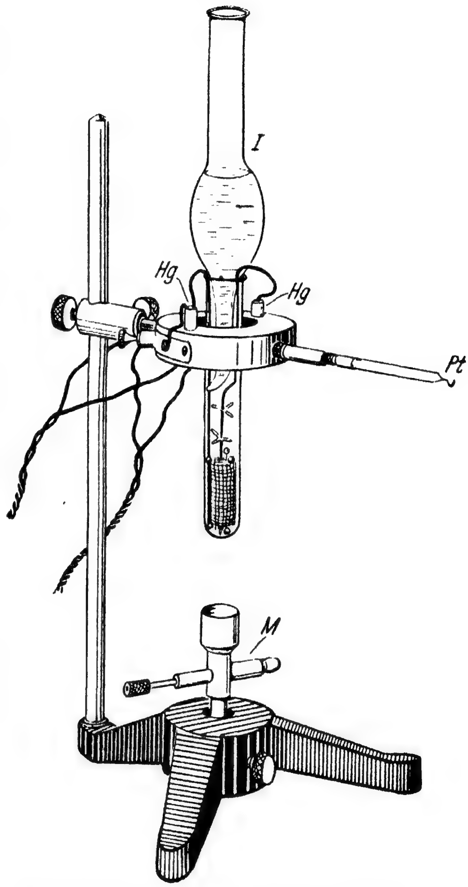

FIG. 64. Micro-electrodeposition of copper. One-third actual size.

Determination of Metals by Micro-Electrodeposition

This method is specially useful if the metal cannot be determined by the above methods, or if only small amounts are present in the organic material, *e.g.*, copper in preserved vegetables. It may also be used to determine the halogens in presence of one another; the sum of the weights of the silver halides is first determined, and the precipitate is then dissolved in 3–4% potassium cyanide solution, and the silver separated electrolytically ; the proportions of chlorine and bromine present can then be calculated.

The maintenance of conditions necessary for the rapid determination of copper on the cathode has proved to be much simpler than was expected even for small amounts of copper, by keeping the liquid in motion by vigorous boiling instead of by the use of a stirrer. It is necessary, however, to ensure that electrolysis is continued until the

liquid has cooled completely in the closed electric circuit, as otherwise the deposited copper redissolves in the sulphuric acid.

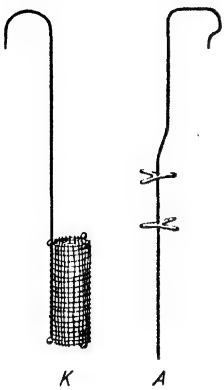

FIG. 65. Platinum cathode (K) and anode (A). Two-thirds actual sizes.

Apparatus (Fig. 64)

The Cell is a simple test-tube (external diameter, 16; length, 105 mm.) which is held in a ring of hard rubber by means of metal springs. The ring is clamped to a stand, and can be adjusted laterally and vertically. The terminals of the electrodes are bent over and dipped in the small mercury contacts, *Hg*, in the rubber ring.

The Cathode is a platinum gauze cylinder (K, Fig. 65), 10 mm. in diameter and 30 mm. long, and a stout platinum wire 100 mm. long is welded to it. In order to avoid contact of the electrode with the wall of the containing vessel during withdrawal, three glass beads (diameter, 1·5 mm.) are fused on to the upper and lower edges. The so-called " enamel " is not suitable for this purpose, as it dissolves appreciably on boiling the liquid during electrolysis.

The Anode (A, Fig. 65) is a platinum wire, bent as shown. To it are sealed two Y-shaped glass pieces to ensure that it takes up a central position inside the cathode and does not touch it during withdrawal. The electrodes should be so arranged that they may be inserted in the test-tube without touching one another.

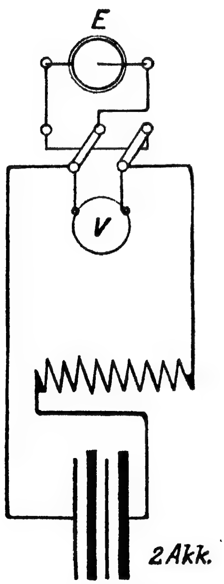

FIG. 66. Circuit for micro - electro - deposition of copper. E, cell. V, voltmeter. Akk, accumulators.

During the first experiments it was found that small losses occurred through the spirting of the liquid, or even through drops of liquid adhering to the wall of the empty portion of the cell. This is overcome by placing in the mouth of the cell a loosely fitting internal condenser (I, Fig. 64), with a bent tip ; it is made from an ordinary test-tube by blowing a bulb in the middle and drawing out the closed end, as shown. After carefully removing any grease from its outer surface with chromic-sulphuric acid, the condenser is filled with water.

The Source of Current is two 2-volt accumulators, in series. In the circuit are an adjustable 6-ohm resistance, a commutator, and a 10-volt voltmeter (V, Fig. 66) ; E is the electrodeposition cell.

Procedure

Copper. The sample is decomposed either in the pressure tube with concentrated nitric acid (p. 97), or in the small Kjeldahl flask (p. 79), with concentrated sulphuric and nitric

acids and perhydrol. The nitric acid must always be completely driven off finally by heating with sulphuric acid until sulphur trioxide fumes appear. The sulphuric acid is then heated over an open flame, air being blown into the flask ; the solution is cooled, diluted with water, boiled strongly for some time to drive off nitrosyl sulphuric acid and nitrous vapours, and rinsed quantitatively into the cell, which has been cleaned with hot sulphuric-chromic acid and rinsed with water. The total volume of liquid should not exceed 7 ml.

The platinum gauze electrode, whether covered with copper or not, is dipped successively in concentrated nitric acid, distilled water, alcohol, and finally in pure ether, and then dried about 1 mm. above a Bunsen flame for 3–5 secs. The terminal of the electrode is heated for a short time in the flame to remove drops of mercury adhering to it. The electrode is cooled by hanging it on a platinum hook, *Pt*, sealed into a glass rod on the apparatus (Fig. 64), and weighed after 5 mins. (on account of the small heat capacity and high conductivity of platinum) on the left balance pan, on which it rests on the three glass beads. First the cathode, then the anode, are introduced into the cell, and their terminals are dipped into the corresponding mercury contacts. The condenser is filled with cold water, and inserted so that its tip is in contact with the wall of the cell. The circuit is closed, the voltage is adjusted to 2 volts by means of the resistance, and the cell is heated by a micro-burner. The oxygen evolved at the anode ensures that the liquid boils vigorously without bumping. A perforated sheet of mica over top of the cell protects the upper portion from the heat. Should the voltage alter during the analysis, it must be readjusted to 2 volts by means of the resistance. In 10–20 mins. it may safely be assumed that the last traces of copper have been deposited on the electrode ; this may be confirmed by testing the solution with potassium ferrocyanide.

The electrolysis is completed by immersing the cell in a beaker of cold water, the current being maintained, and adding more cold water after a few minutes. When the liquid is quite cool the condenser is removed, and, after carefully washing the hands, the anode is held in one hand and the cathode in the other, so as to avoid any lateral movement while removing them from the cell. The cathode with its copper deposit is cleaned, dried, and weighed as before. When the copper deposit was redissolved and redeposited, the recovery was reproducible to within 0·002–0·005 mgm.

Notes. Benedetti-Pichler [8] replaced the sulphuric acid by dilute nitric acid, thus making it possible to dissolve alloys in nitric acid, to dilute to the mark in a large graduated flask, and then to use 1/100 or 1/500 of the whole for the analysis. The following conditions must then be observed :

1. If the concentration of the nitric acid is high, a crystal of potassium sulphate must be added ; and if this is not sufficient, ammonia must be added, drop by drop, until deposition of the copper commences.

2. The somewhat higher voltage of 2·7–3·1 volts must be used.

3. One drop of alcohol must be added at the beginning of the electrolysis, in order to avoid loss by spray due to evolution of gas at the anode. During electrolysis the cathode should project to a height of 3–4 mm. above the liquid, in order that the walls of the cell may be washed down with 1% nitric acid 5 mins. after starting. After a further 20 mins. electrolysis is complete. At the beginning the electrolyte is warmed until it almost boils, and the experiment is so arranged that the bath cools completely in 20–30 mins. The condenser, therefore, is not used.

The anode is first removed, and if it is coated with a deposit of lead peroxide this is removed by placing it in a test-tube containing nitric acid to which oxalic acid has been added; the cathode is then removed and rinsed with distilled water only; if the copper has been deposited in a spongy condition it easily becomes detached if the cathode is rinsed with alcohol. The washed electrode is dried for a short time above a Bunsen flame, and weighed after cooling for a few minutes. The results obtained with brass filings, gun-metal, red brass filings, bearing metal, bronze filings, and metallic dusts of various origins were in good agreement with the duplicate results of macro-analysis. This method is preferable to electrolysis in a solution of pure sulphuric acid, in that considerable amounts of other metals (especially iron) do not interfere.

For all this, the method as described is liable to interference from many other metals which may be deposited with the copper. For use in such cases Lindsey [9] has adapted the well-known macro-method of controlled potentials to the micro-scale. The electrodes and deposits are dipped successively into water and acetone prior to drying, 10 drops of 2% hydroxylamine hydrochloride solution are added as depolariser, and hydrogen is used for stirring; the current strength should fall from 100 to 10 milliamps. in 15 mins. Good results were obtained for the determination of copper in chloride solutions in presence of tin, lead, zinc, aluminium or nickel; antimony is co-deposited with the copper, but the mixed deposit can be re-dissolved in a mixture of hydrochloric and nitric acids, when the copper alone can be re-deposited in presence of chromate ions. The best results are obtained at 60°–70° C., in presence of 15 ml. of solution containing 1 ml. of hydrochloric acid, the anode to cathode potential being 1·2 volt. The deposited metals are conveniently determined colorimetrically (p. 221), instead of by weighing.

Mercury [10] is determined in the apparatus described for copper (Fig. 64), but with a gilded platinum electrode. Thus, 50 mgm. of pure gold foil are dissolved in aqua regia and repeatedly evaporated to dryness on the water-bath after fresh additions of distilled water. The residue is dissolved in 5 ml. of water, containing 0·65 gm. of pure potassium cyanide and electrolysed at 3·5 volts at 55° C. for 2 hrs.

Since wet combustion is not generally suitable, the sample (3–8

mgm.) is decomposed in the pressure tube, to which it is transferred from the weighing tube with the long handle ; 10 drops of concentrated nitric acid (sp. gr., 1·41) are added, and the material is decomposed in the furnace at 270°–280° C. for 2 hrs. After cooling, the condensed drop of liquid in the capillary is driven out by warming, and the tube is opened in the usual manner. The contents of both portions of the tube are quantitatively rinsed into the cell, the total quantity of liquid thus collected being about 5 ml. The electrodes are now inserted and electrolysis is carried out for 40 mins. at 3·5 volts, the cell being immersed in a beaker of water at 40° C. The warm water is replaced by cold water, and after a further 5 mins. the two electrodes are removed from the cool electrolyte without breaking the current. The gauze cathode is now washed in water, alcohol and ether, and dried, without warming, by waving it in the air. Finally, the curved support is passed quickly through a flame twice, and the electrode is weighed after 5 mins. It is necessary to allow for the zero-point deflection of the balance, as the smallest deviations affect the result very considerably. The accuracy of the method, however, is so high that the probable errors do not exceed $\pm$ 0·005 mgm. It must also be noted that mercury deposited in this manner can be removed only by gentle ignition of the cathode, and not completely by immersion in concentrated nitric acid.

Arsenic may also be determined electrolytically on the micro-scale, although one of the methods described on p. 113 is usually preferred. Torrance [11] describes the use for this purpose of an adaptation of the Reinsch test ; this enables arsenic to be determined in presence of copper. Thus 0·3 gm. of sample is heated in a mixture of 10 ml. of nitric acid (sp. gr., 1·42), and 5 ml. of sulphuric acid (sp. gr., 1·82) until fumes appear ; the solution is cooled, 0·2 gm. of hydrazine sulphate is added, followed after evaporation to fuming, by dilution to 1 litre. Under these conditions, electrolysis of 5 ml. of the solution in presence of 1·5 ml. of hydrochloric acid (sp. gr., 1·16) and 6 drops of 50% hydrazine hydrate solution at 65°–70° C. (initial current, 0·1 amp. at 9 volts) for 5–10 mins., completely deposits the copper only. If another portion of the original solution is now reduced by heating it with 1 ml. of a saturated solution of sulphur dioxide, in a corked test-tube in boiling water for 5 mins., electrolysis in the above manner deposits both the copper and the arsenic completely. The latter may then be found by difference. Park [12] deposits the copper selectively in presence of tartaric acid, and determines the arsenic in the residual solution.

Other Methods. Where the quantities of solution for electrolysis are very small (*e.g.*, 1 drop to 3 ml.), the experiment is carried out (according to Donau [13]) in a shallow cup-shaped platinum cathode, the anode being a horizontal coil of platinum wire. Electrolysis is carried out at 2–3 milliamps. (to avoid spirting losses) for 10–20 mins. ; heat is not essential.

It should be pointed out that electrolytic methods other than electrodeposition (and especially potentiometry) have been adapted to use on the micro-scale. As, however, these are applied mainly to problems of inorganic chemistry and involve no new analytical principles (but only an adaptation of existing apparatus to the micro-scale), they will not be dealt with here. Those interested should consult a paper by Borsook and Dubnoff [14] which describes a micro-potentiometric apparatus using the glass electrode. Attention should also be drawn to the polarograph, which enables very small quantities of certain organic compounds which undergo reduction or oxidation at a dropping mercury electrode to be determined; its general applications to organic microanalysis are reviewed by Müller,[15] and special containers to hold about 5 drops of sample are now available.[16]

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CHAPTER IV

DETERMINATIONS OF THE GENERAL GROUPS

DETERMINATION OF CARBOXYL

History and Principles

PREGL'S experience of the titration of ammonia in the micro-Kjeldahl method, and of the preparation and preservation of dilute standard solutions, was the basis of his methods for the determination of equivalent weights. His device for repressing the hydrolysis of the sodium salts of weak acids by means of 50% alcohol normally permits the determination of equivalent weights of up to about 600 with an error of only $\pm$ 0·5%.

Slightly soluble compounds (*e.g.*, bile acids, saponins, etc.) are dissolved in pure, boiling methyl or ethyl alcohol which has been distilled over solid potassium hydroxide, and are titrated after dilution with CO_2-free water. Substances which are re-precipitated even on the addition of a few drops of water cannot be so titrated unless their alkali salts re-dissolve. The use of pyridine or acetone as solvent masks the end-point, but the 50% alcohol is satisfactory ; 65% alcohol can be used if necessary, because with practice the end-point is still sharp with 1 drop (0·02 ml.). With higher concentrations of alcohol the end-point is assessed with certainty only to 2 or 3 drops. With colourless solutions phenolphthalein, or any other indicator which gives a sharp end-point, may be used. Thymolphthalein (pH 9·3–10·5) is very suitable for the titration of yellow to red solutions ; the end-point (colourless to bluish-green) is as sharp, in daylight, as that of phenolphthalein. Deeply coloured substances can be determined by direct titration only if the formation of their sodium salts occurs with a marked loss of, or change in, colour ; potentiometric titration is otherwise preferable.

Lactones may also be determined quantitatively by titration ; some are hydrolysed only by long boiling with excess of alkali, but others react as readily as organic acids. Phenolic hydroxyl groups (*e.g.*, in picric acid), and enolic hydroxyl groups (*e.g.*, in ascorbic acid, vitamin C) may also sometimes be determined in this way. If, however, ascorbic acid is boiled with an excess of alkali, a larger consumption of alkali occurs, owing to the partial splitting of the lactone ring. In the cold, and even in warm aqueous alcohol, on the other hand, one of the two enolic hydroxl groups can be determined accurately. Amino acids are determined (by Grassman and Heyde [1]) by titration with alcoholic sodium hydroxide (*cf.* formol titration, p. 133). With natural products of unknown constitution it is most desirable to check the consumption of alkali in the cold, in the warm and also after boiling with excess of alkali.

Interference by most lactones, alcohols and phenolic hydroxyl groups is eliminated by the method of Tsurumi and Sasaki,[2] in which the organic acid is allowed to react with a solution of potassium hydrosulphide saturated with hydrogen sulphide. Even weak organic acids liberate hydrogen sulphide immediately under the conditions described, and this gas may be determined ; each carboxyl group is equivalent to 1 mol. of H_2S, but the method must be operated with care, and should be used only when the Pregl method fails.

Apparatus

This is described fully on p. 29 (Fig. 25).

Reagents

Hydrochloric Acid (0·01 *N*). Fifty ml. of the 0·1 *N* acid (standardised against sodium carbonate) are diluted to 500 ml. with boiled water (free from carbon dioxide) in a measuring flask, and checked against 3–4 mgm. of pure sodium carbonate. If the acid is carefully prepared, the factor does not alter even after several months.

Sodium Hydroxide (0·01 *N*) is prepared by Pregl's "approximation method" [3] from Sörensen's "oily lye" ; this is made most simply and rapidly by dissolving tablets of pure sodium hydroxide in an equal weight of water, with constant shaking, in a bottle closed with a rubber stopper. This becomes very hot, and it is then placed in water at about 80° C. On account of the lower viscosity at the high temperature, the insoluble sodium carbonate often takes about 6 hrs. to settle.

In the supply bottle for the burette (Fig. 25, p. 29) are placed 400 ml. of boiled distilled water and about 0·3 ml. of the clear "lye" ; the bottle is closed with a cork and shaken. Then 5 ml. are pipetted out and titrated with the 0·01 *N* hydrochloric acid (phenolphthalein as indicator) the liquid being boiled thoroughly before the end-point is reached. The calculated correction is then made to within 10% by the addition of boiled distilled water, and the process repeated. The final correction is made after the solution has stood for 24 hrs., preferably by the potassium bi-iodate method (Zacherl and Krainick [4]). The bi-iodate, $KH(IO_3)_2$, is prepared according to the method of Kolthoff [5] ; it is, however, obtainable pure and is stable indefinitely. Its high molecular weight makes very accurate determinations possible. Since 1 ml. of 0·01 *N* solution contains 3·8995 mgm. of potassium bi-iodate, the amount of iodate weighed out divided by 3·8995 gives the amount of 0·01 *N* alkali required theoretically for the titration.

Under the conditions of titration described below, non-volatile organic acids are also suitable for determining the factor, as well as for checking the alkali. Phenolphthalein or thymolphthalein (not methyl orange) is used as indicator. Thus, benzoic acid is used for the standardisation of very dilute solutions of alkali for the determination of acetyl or benzoyl groups ; if the weight of benzoic acid taken is

divided by 1·2205, the theoretical volume in ml. of 0·01 N alkali required for the titration is obtained.

Phenolphthalein Solution. 1 gm. of phenolphthalein solution is dissolved in 100 gm. of 96% alcohol.

Thymolphthalein Solution. 0·1 gm. of solid is dissolved in 100 gm. of 96% alcohol.

Procedure

From 3–9 mgm. of the sample are weighed into a clean 100-ml. quartz or steamed-out glass flask.[4] If the substance is not readily soluble, it is first powdered finely in an agate mortar. Oily and volatile substances are weighed out in small boats (p. 18) or in micro-weighing bottles (p. 97).

Titration by Pregl's Method. To 10 ml. of pure absolute alcohol and 10 ml. of water are added 2 drops of phenolphthalein; the mixture is heated to the boiling-point and titrated with 0·01 N NaOH until a pink coloration is just produced. It is then decolorised with 0·1 ml. of 0·01 N HCl, boiled for 30 secs., and then titrated at the boiling-point with 0·01 N NaOH until a faint pink coloration is again produced. Two to 4 ml. of this neutral 50% alcohol are added to the weighed substance in the quartz flask, the mixture is warmed if necessary, and titrated with 0·01 N NaOH until a pink colour is just produced; 0·1 ml. of 0·01 N HCl is immediately added and the liquid is boiled for 30 secs. to expel all carbon dioxide. The liquid is then immediately titrated with 0·01 N NaOH until the first pink coloration appears and persists for at least several seconds. Workers with a normal sense of colour can carry out this determination quite correctly after a few failures.

Direct Titration. Pregl emphasised the fact that accurate, freshly prepared solutions use up the same volumes on titration in the cold as after boiling with acid. It is therefore preferable to protect the prepared solutions and solvents most carefully from carbon dioxide and to carry out the titration directly with alkali, allowing the drops to follow one another quickly as in the acetyl determination (p. 161). The accuracy is comparable with that of the above method; only one titration is required; and since the decolorisation occurs gradually, only carelessness makes necessary a back-titration with 0·01 N acid.

For repression of the hydrolysis about 20 ml. of methyl or ethyl alcohol are boiled for 10 mins. with 2 gm. of solid potassium hydroxide under a reflux condenser, in a steamed-out 100-ml. conical flask, which is protected from atmospheric carbon dioxide by a soda-lime tube; the alcohol is distilled off and similarly protected. It is stable for 2–3 days, and when mixed with the same volume of boiled water under the conditions of analysis it gives a definite pink coloration on the addition of 0·02 ml. of 0·01 N NaOH, with phenolphthalein as indicator. The water used is freed from carbon dioxide by boiling it gently for some time over a small flame.

To the weighed sample in the quartz flask are added 3 ml. of alcohol from a pipette, and the flask is closed immediately. To dissolve the substance, the flask is waved over a small flame, and, if necessary, the contents are just boiled ; if it has not dissolved completely in 10 secs. a further 1–2 ml. of alcohol are added, and the boiling is repeated. If this is not effective a second sample is pulverised finely in an agate mortar and used. With certain organic acids which dissolve only after long boiling, it is advisable to note the time from the beginning of the boiling until complete dissolution.

The flame is now removed from the boiling water, 3 ml. of which are pipetted into the flask containing the dissolved substance. The flame is then replaced to keep the water boiling gently, and the titration carried out immediately, 2–3 drops of indicator being added to the solution. Several drops of alkali are run in at first in quick succession, while shaking round and continuously observing the decolorisation described above. If the titration is carried out in the cold, the flask is closed with a soda-lime tube and cooled in a stream of tap-water. As the end-point is approached, half-drops (0·01–0·02 ml.) are added by applying a slight pressure to the upper half of the sphere. If a pink colour appears, the tip of the capillary outlet is touched with the surface of the liquid to be titrated (to remove the alkali still adhering to it), and the solution shaken round. If the colour still remains after 3 secs., the titration is complete. After emptying the flask and rinsing with water, it is ready for the next determination.

Calculation

$$\text{Equivalent weight} = \frac{\text{mgm. of substance} \times 100}{\text{ml. of } 0{\cdot}01\ N\ \text{NaOH}}.$$

$$\text{og (equiv. wt.)} = \log\text{ (mgm. substance)} + 2 - \log\text{ (ml. } 0{\cdot}1\ N\,\text{NaOH)}$$

Examples of Direct Titration :

Cholic acid, $C_{24}H_{40}O_5$, $\frac{1}{2}H_2O$.
8.496 mgm. are equivalent to 2·04 ml. of 0·01 N NaOH.

$$\text{Equivalent}\begin{cases}\text{Theory, } 417{\cdot}56.\\ \text{Found, } 416.\end{cases}$$

Mannonic acid, $C_6H_{10}O_6$.
(*a*) 8·702 mgm. are equivalent to 4·92 ml. of 0·01 N NaOH.
(*b*) 8·815 mgm. are equivalent to 5·01 ml. of 0·01 N NaOH.

$$\text{Equivalent}\begin{cases}\text{Theory, } 178{\cdot}14.\\ \text{Found, } 177\ ;\ 176.\end{cases}$$

Picric acid, $C_6H_3O_7N_3$. (Indicator, thymolphthalein.)
8·533 mgm. are equivalent to 3·71 ml. of 0·01 N NaOH.

$$\text{Equivalent}\begin{cases}\text{Theory, } 229{\cdot}05.\\ \text{Found, } 230.\end{cases}$$

Ascorbic acid, $C_6H_8O_6$.

(*a*) 8·385 mgm. are equivalent to 4·72 ml. of 0·01 *N* NaOH.

(*b*) 8·534 mgm. are equivalent to 4·84 ml. of 0·01 *N* NaOH.

$$\text{Equivalent}\begin{cases}\text{Theory, } 176{\cdot}12.\\ \text{Found, } 177\ ;\ 176.\end{cases}$$

Determination of the Carboxyl Groups of Amino Acids

The method of Willstätter and Waldschmidt-Leitz [6] has been developed as a micro-method by Grassman and Heyde.[1] The titrations are carried out in a 0·01 *N* solution of sodium hydroxide in 90% alcohol, with thymolphthalein as indicator ; the first pale blue colour, not the first colour change, is taken as end-point, and it has been found necessary to carry out the titrations to a colour standard because the end-point is not sharp. An 0·002 *M* solution of cupric chloride in excess of ammonia is used for comparison.

It is best to titrate in a box lined white inside, by the light of a 200-candle power " daylight " lamp. The above end-point necessitates more than the theoretical amount of alkali, and it depends on the amount of alcohol used. It makes no difference, for a given volume of alcohol, whether an amino acid or a mineral acid is being titrated ; to complete the experiment, therefore, a blank test is made with the same amount of alcohol, and the volume of 0·01 *N* NaOH used is subtracted from the volume found in the analysis.

Procedure

The sample is weighed into a graduated 1-ml. flask, having a ground-glass stopper, and dissolved. An aliquot portion (*e.g.*, 0·2 ml.) is transferred to a small flask by means of a fine capillary pipette, divided into 0·001 ml. ; 2 drops of an alcoholic 0·1% solution of thymolphthalein are added, and the 0·01 *N* alkali is run in until a pale blue colour results. Then a 9-fold volume of absolute alcohol (*e.g.*, 1·8 ml.) is added from a pipette ; the blue colour disappears. The titration is finished by adding more alkali until a definite pale blue coloration results.

This method gives very accurate results ; it is very suitable for the determination of small amounts of amino acids, if these are not in an extremely dilute solution. With 0·01 *N* solutions, the volume of alcohol to be added is 10 times that of the solution taken for analysis. With more dilute solutions the concentration of alcohol may be reduced to 80% ; according to Harris [7] this suffices to repress almost completely the hydrolysis of the alkali salts of amino acids.

Formol Titration. This well-known macro-method was devised by Sörensen [8] for the titration of amino acids ; formaldehyde is added to inhibit the effect of the amino group. It has also been applied on the micro-scale, but with phenolphthalein (the usual indicator) the results are not very accurate, especially if the solution is at all coloured. Janke and Mikschik [9] have obtained better success by a potentiometric

method. A bulb-shaped micro-glass electrode is used, electrical contact being ensured by a layer of silver on its surface; 0·1 ml. of solution can be titrated.

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DETERMINATION OF ACTIVE HYDROGEN

History and Principles

Tschugaeff [1] observed in 1902 that the magnesium alkyl halides of low molecular weight react with carboxyl and hydroxyl groups, with evolution of gas. Zerewitinoff [2] pointed out later that the corresponding hydrocarbon is thereby formed, and he used this reaction for the quantitative determination of active hydrogen. Hibbert and Sudborough [3] pointed out experimental difficulties which arise from the great capacity of the magnesium methyl iodide for reaction with oxygen and water. Flaschenträger [4] adapted the method to the micro-scale. The substance is dissolved in an inert solvent and is treated with magnesium methyl iodide; the liberated methane is collected.

$$RH + CH_3MgI \rightarrow RMgI + CH_4.$$

Difficulties arise owing to the extremely unpleasant properties of the Grignard reagent. Thus Meisenheimer and Schlichenmayer found that it is essential to work in an inert atmosphere,[5] *e.g.*, nitrogen, containing less than 0·2% of oxygen [6]; a source of error which produces low results is thus completely eliminated. A further condition is the complete absence of moisture from the apparatus and reagents. Details for the drying of the apparatus and the nitrogen are given later.

Even with careful exclusion of oxygen and water, the micro-modification of the method is still subject to an error which depends on the amount of Grignard reagent. The small amounts of residual methyl iodide in the Grignard reagent is the source of the small blank value, which increases considerably on warming the reaction flask above 50° C. It has been shown, however, that these traces may be removed in an atmosphere of nitrogen by reduced pressure at 50° C.; the reagent so treated has proved quite satisfactory.

It is also evident that the reaction can be quantitative only if the substance to be analysed is completely dissolved, and the few solvents which can be used (*iso*amyl ether, xylene, anisole or pyridine) must be of such a degree of purity and dryness that there is no evolution of gas in the blank test. Of these, anisole, and, especially pyridine, are

suitable on account of their low vapour pressures and high solvent powers. The usual method of drying anisole over sodium is inadequate ; if, however, it is shaken with and stored over phosphorus pentoxide, the last traces of moisture are removed. Since many substances only dissolve in warm or boiling anisole, it is sometimes advisable to dissolve the substance in a mixture of a few drops each of those solvents and to subtract the value of the blank due to the pyridine from the volume read. Recently, pyridine has been purified over perchlorate and carefully dried, and it then answers all requirements and evolves no gas in blank tests. If it is necessary to carry out the reaction at temperatures above 50° C. the blank test must be made at the same temperature.

Pyridine is considerably better as a solvent than is anisole, and it is now used for sugars, many sterols, etc., which dissolve very readily in warm pyridine. A further advantage of pyridine arises with substances which are decomposed by warming with anisole (*e.g.*, alkannin). With pyridine, the reaction is almost always complete at room temperature, whilst with anisole (particularly in presence of several active hydrogen atoms, *e.g.*, as in cholic acid) the reaction is complete only at 95° C. In pyridine at room temperature, one atom of hydrogen in hydroxyl, hydrosulphide and carboxyl groups and two in water of crystallisation, are active ; one hydrogen atom of acetamide reacts in the cold, two at 95° C. ; in urea, two react in the cold and 3·5–3·8 at 95° C. in 5–8 mins. ; aniline has one active hydrogen, both in the cold and on warming ; *o*-phenylenediamine has two under both circumstances ; acetanilide has one active in the cold.

With azo-benzene, the ethane evolved may be determined either at room temperature or after heating to 95° C. If nitro- or sulpho-groups occur in the structure of the ring, as in picric acid and sulpho-salicylic acid, respectively, these are not affected by the Grignard reagent at room temperature ; on warming, however, evolution of gas occurs and makes the result useless. The halogen atoms in *o*-chloro-benzoic acid, *m*-bromobenzoic acid, and *p*-iodobenzoic acid are not affected by the Grignard reagent, either hot or cold ; one molecule of methane is formed from the carboxyl group only. With an unsaturated lactone (carolic acid [8]) the Grignard reaction was negative in anisole solution ; in pyridine, 1 molecule of methane was obtained. The authors [8] explain this as due to an enolic transformation with pyridine as solvent, and it therefore is advisable to keep anisole available as well as pyridine.

Lüttgens and Negelein [8] have checked the method, using anisole as solvent, by the manometric technique of Warburg (*cf.* p. 176) and they obtained excellent results even with weights of 0·9 mgm. For an analysis without heating 25–30 mins. are required after some experience ; the error is ± 3%.

In a modification of the above volumetric method [9] the methane evolved is burned to carbon dioxide and water, which are absorbed and

weighed. In another, which has obvious restrictions in its applications, a solution of the substance in deuterium oxide is evaporated to dryness, when the active hydrogen is replaced by the deuterium ; this produces an increase in weight which is determined gravimetrically. Similarly, the method may be used to distinguish between labile and active hydrogen, *e.g.*, as in the methylene group of malonic acid.[11]

Apparatus

The Jena-type glass reaction-vessel (Fig. 67) has a capacity of about 17 ml. Complete air-tightness is ensured by a ground-in joint 25 mm. long, which is secured with two steel springs. The nitrogen inlet-tube (bore, 2 mm.) and the delivery tube for nitrogen or methane (bore, 1 mm.) are sealed into the hollow stopper. The cock H_a serves to close the system after the displacement of air. The inlet tube is forked at the end exactly as shown, to ensure rapid displacement of the air. Air-tightness of the inlet tube is ensured by connexions of glass in contact with glass, inside pressure-tubing which has been treated *in vacuo* with paraffin wax.

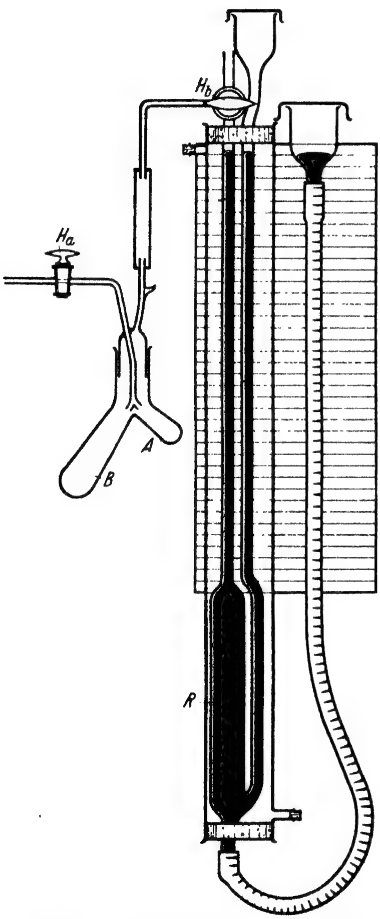

Fig. 67. Micro-determination of active hydrogen.

The Grignard reaction is carried out in the limb B, the shape of which prevents moistening of the stopper on shaking. The short limb A holds 2·5 ml. of the Grignard solution, with the vessel in the vertical position. An inclination of about 60° brings all the Grignard reagent into reaction with the substance in limb B.

The micro-burette is connected with the reaction vessel by a small side-tube bent downwards at right angles, and by a 70-mm. piece of treated tubing (p. 39). The nitrogen or methane enters the burette through the three-way cock H_b (or a T-piece with stopcocks). The burette is 37 cm. long ; is graduated in 0·01 ml., and has a capacity of 4 ml. For reactions at higher temperatures the reaction-vessel must first be warmed up to 95° C. In order that the excess pressure thus set up may be reduced on lowering the level of the mercury, a 10-ml. gas or mercury reservoir,

R, is sealed on at the end of the burette ; the comparison tube branches off at the lowest point of *R*. Mercury is used as sealing liquid, and a jacket filled with water surrounds the entire burette.

When reading the volume of gas the three mercury levels are brought to the same height ; a lined card placed behind the burette facilitates estimation to 0·001 ml. Since the volume of methane read is very small in proportion to the volume of the reaction-vessel, the readings of the mercury must be made at exactly the same temperature (*e.g.*, room temperature) before and after the determination. A container holding 20–30 litres of water is kept in the laboratory and is used to fill the reaction vessel before and after the analysis.

Reagents

Nitrogen. Cylinders of very pure nitrogen are not always at hand in the laboratory, and the purification of commercial nitrogen will be described.

Commercial Nitrogen containing up to 5% *of Oxygen.* To avoid repeated reduction of the copper oxide formed and to allow the gas to remain in contact with the copper as long as possible, an ordinary combustion tube (bore, 23 mm.) is used. This is charged with pieces of reduced copper (in wire form) to a length of 50 cm. ; two layers of copper oxide in wire form, 5 cm. long, are held in place by a spiral of wire gauze on each side, and the tube is heated at dull red heat in a combustion furnace. For further purification the gas is led through a wash-bottle containing 50% alkali, and it is then dried in a similar bottle containing concentrated sulphuric acid ; the last traces of moisture are removed in a U-tube filled with phosphorus pentoxide.

Nitrogen of 99·5% *Purity.* A macro-combustion tube is cut down to 35 cm. and filled with a 15-cm. layer of pieces of reduced copper wire ; two short copper spirals enclose this layer. The tube filling is kept glowing on a micro-combustion stand by means of a micro-burner. The further purification and drying are as described above.

Nitrogen of 99·9% *purity*, as used for filling electric bulbs, can be purchased ; purification is unnecessary. The nitrogen is led directly from the bomb, through the drying vessels, into the reaction vessel.

Storage of the purified nitrogen in a gasholder is impracticable ; the nitrogen absorbs atmospheric oxygen from the water seal even after a few days. After completion of the analysis the apparatus for purifying and drying the nitrogen is stored in nitrogen and protected against moisture by a small guard-tube of phosphorus pentoxide.

The purification of the other reagents is described below.

***Iso*amyl Ether** is allowed to stand for a few days over sodium, and is then distilled over fresh sodium (b.p., 171°–172° C. at 750 mm.).

Magnesium. Pieces of ribbon, 30 mm. long, are cleaned successively with dilute acetic acid, alcohol, and ether. Commercial shreds form a fine sludge, which obstructs the pores of the fritted glass plates during suction.

Methyl Iodide. Commercial methyl iodide is purified by fractional distillation (b.p., 43° C.).

Anisole is distilled from and stored over sodium (b.p., 152·5°–153·0° C. at 761 mm.).

Pyridine. 500 gm. are freed from homologues over perchlorate by the method of Arndt and Nachtwey.[12] The product is distilled *in vacuo*, and shaken mechanically for 6 hrs. with pieces of barium oxide in a strong bottle with a ground-in stopper. If solid barium oxide is not present after shaking, more is added, and after further shaking the mixture is filtered quickly through a folded filter into a bottle with a cap, in which coarse barium oxide is placed. After a few days the pyridine is ready for use.

Grignard Reagent. 50 gm. of *iso*amyl ether, 4·5 gm. of cleaned magnesium ribbon, and 18 gm. of methyl iodide are placed in a dry, 150-ml. round-bottomed flask fitted with a ground-in reflux condenser with a calcium chloride tube. A few crystals of iodine are added to start the reaction and the flask, with the condenser, is placed on a cold water-bath which is then heated. The reaction thus proceeds gradually; if it is too vigorous, the water-bath is removed. After the beginning of the reaction the water-bath is boiled for 30 mins. and then removed.

After cooling to about 30° C. the reflux condenser is replaced by a nitrogen inlet-tube and a ground-in distilling condenser. The excess of methyl iodide is now distilled off for 30 mins. on the water-bath in a stream of nitrogen. Meanwhile a glass suction filter (11 G 4, Schott) and a 100-ml. filter flask are dried in the oven at 110° C. The hot flask, with the filter attached, is connected with a good water-pump, and nitrogen is drawn through the filter from the container. If the flask and filter are allowed to cool in air after removal from the oven, a coating of moisture is formed, which reacts with the reagent. After the Grignard reagent and filter have cooled to room temperature, the turbid reagent is decanted from the residue of magnesium ribbon into the filter, and suction is applied for 30–60 mins. Unnecessary admission of air is avoided if the tubing is removed from the pump while 1–2 mm. of Grignard solution still remain on the filter. Avoiding the fine film formed by shutting off the vacuum, the clear solution is decanted into an absolutely dry 100-ml. flask, with a ground-in stopper, which has been cooled while nitrogen is passed through it.

This reagent still contains traces of methyl iodide, which must be removed. For this purpose a 100-ml. Claisen flask is alternately evacuated and filled with nitrogen ; the filtrate is then placed in the flask and evacuated. Sudden breaks in the vacuum are avoided by means of pinchcocks, and a manometer is used as a check. The remaining traces of methyl iodide are now removed in an atmosphere of nitrogen, under reduced pressure, on a water-bath at 50° C. for 30 mins. While the Grignard reagent is cooling the flask is slowly filled with nitrogen, using a mercury excess-pressure valve. Ten test-tubes are now dried in a drying-oven at 110° C., cooled in an

atmosphere of nitrogen, and closed with tight-fitting rubber stoppers. The Grignard reagent is quickly decanted into these tubes, in 3- to 7-ml. portions, through a small funnel which ends 6 cm. above the bottom of the test-tube. The test-tubes are now sealed with the blowpipe 5–6 cm. above the Grignard reagent, and allowed to cool slowly. Sometimes black rings are formed below the point at which the tube is sealed ; they have no ill effect if, during the opening operation, the bulb is burst immediately above the reagent. The reagent is stable and perfectly clear, and has a yellowish-green tinge. Its strength (1·2–1·4 *N*) is determined by decomposing 1 ml. with an excess of 0·1 *N* hydrochloric acid and titrating back with 0·1 *N* alkali, using phenolphthalein as indicator.

Procedure [13]

Preliminary. Complete solubility in pyridine or anisole of the substance to be analysed, and absence of moisture, are most important for a satisfactory determination. All substances must be very finely powdered and dried in a desiccator over phosphorus pentoxide for several hours. Occasionally alcohol of crystallisation and water of crystallisation are strongly retained by the substance and only given up slowly, so that drying at 100° C. or higher is indispensable.

If anisole is used as solvent, the amount required for the determination is placed in a small bottle with a ground-in stopper, mixed with about one-twentieth of its weight of phosphorus pentoxide, and shaken well several times. On standing the phosphorus pentoxide settles at the bottom, and the clear anisole may be pipetted off. A Grignard reagent tube is filed about 1 cm. above the liquid level, and the tip broken off with a hot glass drop. The contents are poured into a small flask with a ground-in stopper (which has been dried at 115° C. and cooled, while nitrogen is passed through), and the stopper is firmly inserted.

As is usual in volumetric determinations, the amount of substance weighed out corresponds with the volume of gas conveniently measured. If solid the substance is weighed, from the desiccator, into the small weighing tubes with the long handles, and placed at the bottom of limb *B* (Fig. 67). If the substance has been quantitatively dried in a small platinum boat, this is allowed to slide into the limb *B* by inclining the reaction vessel. For weighing oils and liquids, micro-weighing bottles (p. 97) are preferable. Liquids with high vapour-pressures are weighed in a Pirsch capillary (p. 202), and this is pushed, with the tip downwards, into limb *B*, the solvent having been previously placed in the reaction-vessel. By carefully warming in a stream of nitrogen the liquid is driven out of the capillary. The reaction-vessel (cleaned successively with acidified water, water, alcohol, and acetone), the stopper, and the pipettes required are placed in an oven at 110° C. The reducing valve is adjusted so that 100–150 ml. of nitrogen pass in 5 mins.

The reaction-vessel is then removed from the oven, the stopper inserted, secured by a steel spring, and connected with the U-tube and burette ; the slightly greased stopcock H_a is inserted, and the air is displaced from the vessel and burette. While the vessel cools in a current of nitrogen (for 5–7 mins.) the substance is weighed out.

The Reaction. The cool vessel is then removed ; the pipettes are allowed to cool while nitrogen is passed through, and the substance is transferred from the balance to limb *B*. Either 1 ml. of pyridine or 3 ml. of anisole are added to dissolve it ; careful warming in an atmosphere of nitrogen may be used. When the substance has dissolved completely, 0·5 ml. of Grignard reagent is pipetted into limb *A*, the upper half of the stopper is well greased with vaselin, the steel spring is hooked up, and the reaction-vessel is closed as before. The vessel is then suspended in the water-bath and the mercury level is adjusted between the zero and 0·1-ml. marks on the burette. Nitrogen is passed through the apparatus for 5 mins., and the weighing-tube is weighed ; the pipettes are cleaned for the next determination with methylated spirit and acetone (those for the Grignard solution being cleaned successively with water acidified with hydrochloric acid and with water), and placed in the drying-oven. The stopcocks H_a and H_b are now closed, and the current of nitrogen stopped. If a pressure-change is perceptible after 3 mins. atmospheric pressure is restored by opening the cock H_b. After 3 mins. more no further alteration in pressure should occur. If a decrease in volume is observed, this may be corrected by leading nitrogen through again ; it is not usually possible to correct for the excess pressure due to moisture.

The height of the mercury, the barometer reading and the temperature of the water are noted, and the reaction is carried out as follows :

The water-bath is removed, the Grignard solution is allowed to flow on to the substance by tilting the reaction vessel through 60°–70°, and the vessel is shaken round (usually for 1 min.) until no more gas evolution is seen in the burette. Then the excess pressure produced by the evolution of methane is removed by lowering the bulb of mercury, and the reaction-vessel is again placed in the water-bath. After 5 mins. the volume is read to 0·005 ml., with the three mercury levels at the same height. If the reaction is carried out at higher temperatures, the reaction vessel is placed in a suitable water-bath and frequently shaken. After 5 mins. the water-bath is brought to room temperature ; the temperature is read after 10 mins. The vessel is removed, the vaselin cleaned off by benzene, and the apparatus cleaned as before. The reaction-vessel and pipettes are dry again in 15 mins. The first analysis must always be preceded by a test analysis or blank determination. If no more analyses are to be made, the burette must be protected against moisture by a small tube of phosphorus pentoxide. The purity of new reagents must be checked by a blank test.

Calculation

Taking 0·7168 gm. as the weight of 1 litre of methane at N.T.P., the percentage of active hydrogen is given by the equation :

$$\text{Percentage of hydrogen} = \frac{1\cdot0078 \times 100 \times 100v_0}{22365 \times s} \qquad (1)$$

$$= 4\cdot506 \times v_0/s.$$

$$\log (\text{factor}) = 0\cdot65381.$$

$$v_0 = \text{reduced volume in ml.}$$

$$s = \text{weight of sample in mgm.}$$

If the factor f_N of the nitrogen tables is used for reduction to 0° C. and 760 mm. pressure, the calculation of v_0 may be omitted. Accordingly we have :

$$\text{Percentage of hydrogen} = 3\cdot604 \times f_N \times v/s.$$

$$\log (\text{factor}) = 0\cdot55673.$$

If the work has been carried out in pyridine at higher temperatures the value obtained in a blank determination must be considered, as well as the vapour-pressure of the pyridine. For the calculation of f_N, the vapour-pressure of pyridine at the corresponding temperature, given in the following table, must be subtracted from the barometric reading :

Vapour Pressure of Pyridine

(" Jahrestabellen chemischer, physikalischer, biologischer und technologischer Konstanten und Zahlenwerte," 1929, Vol. 9, p. 179.)

t° C.	p (mm).	t° C.	p (mm.)	t° C.	p (mm.)
10	7·7	18	13·4	26	20·8
12	9·0	20	14·9	28	23·2
14	10·2	22	15·7	30	25·7
16	11·8	24	18·6	32	29·0

Examples :

Glucose	t	v_0	Percentage of active hydrogen found
3·145 mgm.	21° C.	1·93 ml.	2·77
1·520 mgm.	21° C.	0·93 ml.	2·76

Glucose, $C_6H_{12}O_6$, contains five active hydrogen atoms per molecule.
Molecular weight = 180·08.
Active hydrogen (calculated), 2·78%.

Sulphosalicylic acid	t	v_0	Percentage of active hydrogen found
2.175 mgm.	22° C.	1·32 m l.	2·73
2·545 mgm.	21° C.	1.56 ml.	2·76

Crystalline sulphosalicylic acid, $C_7H_6O_6S + 2H_2O$, contains seven active hydrogen atoms.

Molecular weight $= 254{\cdot}12$.

Active hydrogen (calculated), 2·75%.

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DETERMINATION OF PRIMARY AMINO GROUPS

Principle of the Method

The Van Slyke method depends on the fact that many primary amino groups react with nitrous acid according to the equation :

$$RNH_2 + HNO_2 - ROH + H_2O + N_2.$$

The nitrous acid is prepared from sodium nitrite and glacial acetic acid. The oxides of nitrogen thus liberated are used to drive all air out of the apparatus, before addition of the material to be analysed. The oxides of nitrogen are absorbed in a Hempel pipette by means of alkaline permanganate solution, and the remaining nitrogen is determined volumetrically in a measuring burette.

With all amino acids and the peptides derived from them the reaction takes 5 mins. at room temperature [2]; with lysine the reaction of the second amino group (in the ϵ-position) is complete only after 30 mins. The nitrogens of the pyrrolidine, indole, and imidazole rings do not react, so that only 50% of the total nitrogen of tryptophane, 33% of that of histidine and 25% of that of arginine is available. No nitrogen is split off from proline or oxyproline or from the guanidine group, $NH_2.C(NH)NH_2$, in free guanidine, or in creatine or arginine. Methylamine and ammonia react on shaking for 90–120 mins.; amino-purine or amino-pyrimidine after 2–5 hrs. Glycocoll, cystine and unhydrolysed cephaline give results which are usually 103% and 107% of the theoretical values, respectively.[3] With urea, the reaction takes about 8 hrs.

Reagents

Sodium Nitrite Solution. 30 gm. of sodium nitrite (A.R.) are dissolved in 100 ml. of water. Small amounts of nitrogen are liberated even from the purest grades by acidification (see p. 145).

Glacial Acetic Acid. A.R. grade is used.

Alkaline Permanganate Solution. 50 gm. of potassium permanganate and 25 gm. of potassium hydroxide are dissolved in 1 litre of water.

***Secondary* Octyl Alcohol.** A.R. grade is used.

Apparatus

This consists (Fig. 68) of three principal parts, a reaction vessel with a burette and funnel for filling it, a measuring burette, and a Hempel pipette.

The Reaction-Vessel (*D*) is cylindrical and has a capacity of about 6 ml. At the bottom it is constricted, and joins a tube with a glass tap (*d*), for running off the solution. At the top the reaction-vessel joins a capillary (bore, 1·0–1·5 mm.), and a bulb between them prevents the liquid from being shaken into the capillary. After about 8 cm., the capillary is bent at right angles and leads to a three-way cock (*c*) connecting to the measuring burette (*F*) and to a lower tube which can draw off the gases from the reaction-vessel or the measuring burette. A capillary bent downwards, and sealed to the cock (*c*) is connected, by rubber tubing, with the Greiner-Friedrich cock (*f*) of the measuring burette.

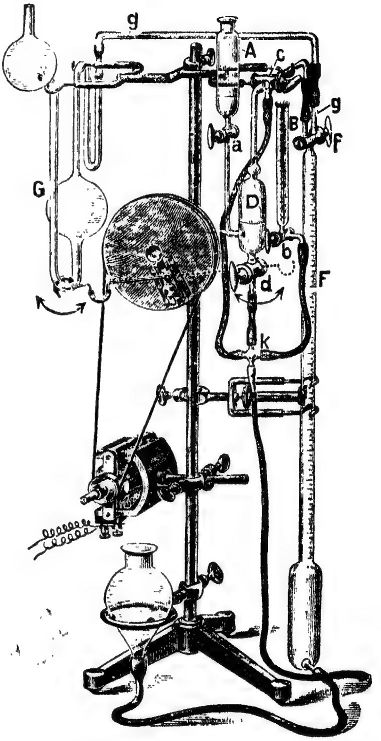

Fig. 68. Van Slyke micro-apparatus for determination of primary amino groups.

Just above the constricted base of the reaction-vessel is the capillary three-way cock (*b*) which connects with the 2-ml. burette (*B*) and with a short tube bent downwards; the burette is subdivided into 0·01 ml. The 14-ml. cylindrical funnel (*A*) with stopcock (*a*) joins the other side of the reaction-vessel at the same height.

The reaction-vessel and funnel have file marks, indicating about 40% and 20% of the volume of the reaction-vessel, respectively. The cock (*d*) of the reaction-vessel is connected with a four-way joint (*K*) by rubber tubing. The horizontal arms connect with the cocks (*b*) and (*c*) by means of rubber tubing, and a long piece of tubing, open at the bottom, is fitted over the lower arm of (*K*).

The Measuring Burette (capacity, 3 ml.; height, about 30 cm.; internal diameter, 3–5 mm.) is subdivided in 0·01 ml. Below the cock (*f*) it narrows to a capillary 2–3 cm. long, in which is the zero-mark.

At the base is a reservoir for the absorption of the reaction gases (and especially the oxides of nitrogen), and this is connected, by rubber tubing, with a levelling bulb. The Greiner-Friedrich cock is connected to the thick-walled horizontal capillary tube which joins the burette (*F*) and the Hempel pipette (*G*).

The Hempel Pipette consists of two glass bulbs, at different heights, each 70 mm. in diameter; the lower is connected with (*g*) by a capillary tube bent twice through 180°.

The whole apparatus is supported on a stand, but only the burette, motor, and pulley are fixed. The reaction-vessel hangs only on two forks at (*c*). The stopcock (*d*) and the lower bend of the Hempel pipette are at the same level; the pulley of the shaking motor is fixed between these parts of the apparatus. A guiding-rod may be placed radially on the pulley, so that the amplitude, and therefore the degree of shaking, may be carefully regulated. The speed of shaker may be further regulated by the controlling resistance of the motor.

Procedure

The substance is weighed into a 2-ml. or 5-ml. graduated flask by means of the nitrogen weighing tube (p. 73), dissolved in water, and diluted to the mark, so that 1 or 2 ml. will yield 0·5–2·0 ml. of nitrogen. It must be remembered that, in this method, an amino group yields twice as much nitrogen as in the Dumas method. If the substance is insoluble in water a solution in 2 *N* mineral acid, 50% acetic acid, or dilute alkali may be used.

The apparatus is cleaned successively with chromic-sulphuric acid, distilled water and alcohol, and dried; the rubber connexions are made with tight-fitting tubing, and the cocks are greased well with high-vacuum fat. The Hempel pipette is then filled with potassium permanganate solution up to the rubber connexion, distilled water is passed into the measuring burette by manipulating the levelling bulb, and both vessels are connected by means of the bridge (*g*), which is full of water; the whole apparatus should be free from air, and on lowering the bulb no air-bubbles should appear. By means of the bulb the permanganate solution is driven through (*g*) up to (*f*), the air from the burette is driven out up to (*c*), and water is again brought into the bridge. The reaction-vessel and the capillary of the burette are then connected through (*f*), and the former is filled with water from the measuring burette up to the cock (*c*), which is then closed against the burette. Before displacing the air, the cock (*b*) under the burette must be closed, and the bore must be empty.

In order to drive out the air with the oxides of nitrogen, glacial acetic acid is poured into the funnel (*A*) up to the mark, and allowed to flow into the reaction vessel (*D*). Similarly, sufficient sodium nitrite solution is added to fill the reaction-vessel to above the bulb; air escapes through (*c*) *via* the four-way tube (*k*). The cock (*c*) is then closed against the reaction-vessel, which is shaken with (*a*) open until

the oxides of nitrogen push the mixture in it down to the mark. The oxides of nitrogen are allowed to escape through (*c*), and the shaking is repeated to remove the last traces of air. After the gas has been drawn off, the solution is again displaced to the mark, (*a*) is closed, and the connexion with (*F*) is made through (*c*) while the levelling-bulb is at its lowest position.

The solution of the substance is now transferred to the burette, and its level is adjusted exactly to the zero-mark by running it out through (*b*). An exactly measured amount of the solution is now drawn carefully into the reaction-vessel by lowering the levelling-bulb, and shaking is begun. The time required for shaking depends on the nature of the substance analysed; usually 3–5 mins. are necessary. With very frothy substances 0·5–1 ml. of octyl alcohol is added to the reaction-vessel from the burette during the removal of the air; the burette must first be well rinsed out with glacial acetic acid.

After the shaking is finished, the cock (*a*) is opened and the mixed nitrogen and oxides of nitrogen are driven into (*F*). The cock (*f*) is turned through 180°, and by raising the levelling-bulb the whole of the gas is driven from the burette into the Hempel pipette. The absorption of the oxides of nitrogen and the carbon dioxide is accelerated by slowly shaking the Hempel vessel for 2 mins.

The residual gas, which is pure nitrogen, is again driven into the measuring burette, the level is adjusted to zero, and the volume is read as usual to 0·002 ml., after levelling to atmospheric pressure. The nitrogen is then discharged through (*c*) into the atmosphere, and the whole determination is carried out again from the beginning of the shaking. If the reaction has been quantitative, the second volume read is no greater than that obtained in the blank determination.

The blank test is made immediately before or after the corresponding analysis, and under exactly the same conditions but without the material to be analysed; the volume of nitrogen so obtained depends not only on the purity of the reagents, but also, within certain limits, on the time of shaking. With good solutions of nitrite, 0·003–0·005 ml. of nitrogen is produced on shaking for 5 mins. The blank test is usually constant for the same reagents, and it is sufficient to check it before every new series of analyses.

Calculation

The difference between the volumes of nitrogen found in the determination and in the blank test is divided by two, and the temperature and barometric pressure are read.

The nitrogen is calculated as in the Dumas method, using the table for the reduction of gas in Küster's logarithmic tables.[4] Since water is used as the sealing liquid, the vapour pressure of water at the temperature of the reading must be subtracted from the barometric pressure.

$$\log(\%\ \text{N present as } NH_2) = \log v + \log \text{N} + 2 - \log(\text{mgm. sample}).$$

Example :

5·496 gm. of alanine were dissolved in 2·0 ml., and 1·0 ml. was used.

From the 2·748 mgm. of alanine, 0·798 ml. of nitrogen was obtained at 21° C. and 757 mm. pressure. After subtracting the blank of 0·004 ml., and dividing by 2, $v = 397$ ml. The partial pressure of water vapour at 21° C. is 18·7 mm. (approx. 19 mm.); log N, at 738 mm. = 0·05210.

Molecular weight of alanine, $C_3H_7NO_2 = 89{\cdot}06$.

$$\text{Nitrogen}\begin{cases}\text{Theory, } 15{\cdot}72\%.\\ \text{Found, } 15{\cdot}55\%.\end{cases}$$

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DETERMINATION OF METHOXYL AND ETHOXYL GROUPS

Two types of procedure are available. Both are based on the Zeisel method, in which the alkoxyl group is split off as the alkyl iodide by boiling the sample with hydriodic acid. In the gravimetric version of the method the volatile iodide is carried over in a stream of carbon dioxide into a solution of silver nitrate in alcohol. The double salt ($AgI,AgNO_3$) so formed is decomposed into silver nitrate and silver iodide by the addition of dilute nitric acid, and the latter is weighed.

In one volumetric modification [1] the alkyl iodide is absorbed in pyridine, and a solution of the resulting compound in water is mixed with potassium chromate; the iodine so liberated is titrated with standard sodium thiosulphate solution in the usual way. In the alternative volumetric method [2] the alkyl iodide is collected in a solution of bromine, sodium acetate, and acetic acid in water, when the following reactions occur :

$$CH_3I + Br_2 \rightarrow CH_3Br + IBr.$$
$$IBr + 3H_2O + 2Br_2 \rightarrow HIO_3 + 5HBr.$$

The sodium acetate neutralises the hydrobromic acid formed, and the iodine liberated from the iodate on addition of potassium iodide may be titrated with sodium thiosulphate solution.

Both types of method give good results, although on the whole the latter volumetric procedure is to be preferred (see p. 152). The method as a whole has been the subject of considerable investigation, and its reliability has been adequately established; the collaborative work organised by the Association of Official Agriculture Chemists [3] may be cited in this connexion.

Apparatus (Fig. 69)

This is made of Jena-type glass, and consists of the olive-shaped

flask *SK* (4–5 ml. capacity) with a vertical exit-tube *SR* and a side-tube *A*, through which the material and the carbon dioxide are introduced. In order to prevent the escape of vapours during the determination, the volume of *A* is reduced by the insertion of a small, loosely fitting glass tube, sealed at both ends, which is prevented from slipping into the flask by a small collar through which the carbon dioxide passes. If the side-tube rests at an oblique angle, any hindrance to the gas current is avoided. The exit-tube *SR* of the boiling flask

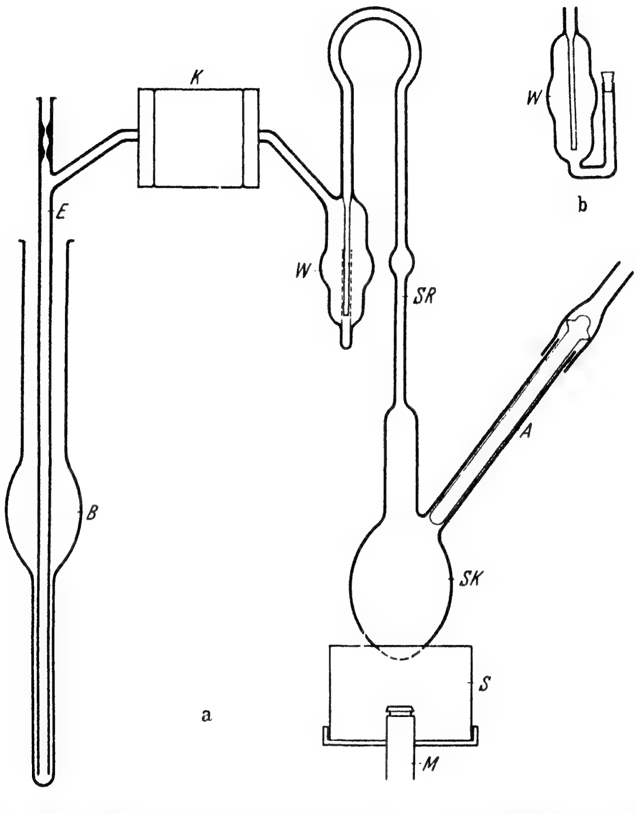

FIG. 69. Micro-determination of alkoxyl groups. (*a*) Apparatus. (*b*) Washer with filling tube. Half actual sizes (flask, *SK*, two-thirds actual size).

widens at about 4 cm. above the flask, into a small bulb, so that the tube serves as an air condenser. At 6–8 cm. above the bulb the tube is bent down and is sealed into the washer W. It is advisable not to fill the washer from below, but to add the washing liquid through the filling tube after the apparatus has been set up.

The washer is connected with the gas inlet-tube *E* by a small tube passing obliquely upwards, then horizontally, and finally slanting downwards ; *E* is open at both ends. Around the horizontal part of the connecting tube a split perforated cork (*K*) is bound with copper wire, to enable the apparatus to be held in a clamp. The tube *E* has

two constrictions just above the point at which it joins the connecting tube, and before using the apparatus a small drop of water is placed over the upper of these. *E* is then closed at the top with a small cork. This water seal makes a simple but efficient joint, which is impermeable to the vapours of alkyl iodides.

A test-tube (*B*) which is widened in the middle, is used as receiver. The narrow lower part has a diameter of 7–8 mm., and is 50 mm. long. In the relatively narrow space between this and the delivery tube the bubbles rise only slowly, so that the alkyl iodide is quantitatively absorbed in the alcoholic silver nitrate solution in *B*. Two millilitres of water are placed in every new absorption vessel, the meniscus is marked, and in the analyses the alcoholic silver nitrate solution is filled exactly to this mark.

Reagents

Hydriodic Acid (sp. gr., 1·7) which has been specially prepared for the determination of methoxyl by the Zeisel method is now obtainable. All conditions which promote the decomposition of hydriodic acid and separation of iodine (*e.g.*, exposure to light and, more particularly, contact with air) should be carefully avoided. Often, after standing for a long time, preparations which became deep brown and opaque yield low values for methoxyl because the concentration of the hydriodic acid falls below that corresponding with sp. gr. 1·7.[4] In order that fresh hydriodic acid is always at hand, only 25- or 50-gm. batches should be ordered.

In exceptional cases it may be necessary to use hydriodic acid of sp. gr. 1·96 (*e.g.*, for the determination of glycerol in aqueous solutions) in order to raise the specific gravity to that of the constant boiling mixture after the addition of strong hydriodic acid. It should be noted that hydriodic acid distils from the flask until the concentration of the constant boiling mixture is attained, and the solution of thiosulphate in the washer may be insufficient to absorb it quantitatively.

Alcoholic Silver Nitrate Solution. A solution of 4 gm. of silver nitrate (A.R.) in 100 mgm. of 95% alcohol in a 200-ml. round-bottomed flask is boiled for 4 hrs. on a water-bath under a reflux condenser. After standing for 2 days the liquid is decanted from any separated silver into a brown stock-bottle.

Friedrich [5] noted that the alkyl iodide is transformed quantitatively into silver iodide only by a freshly prepared alcoholic silver nitrate solution. Since, however, the absorbing power of the silver nitrate solution is practically constant after about a week, he suggests adding 0·12 mgm. to the weight of the final precipitate for every 2 ml. of alcoholic silver nitrate solution used. This gives good results for 6–10 months, but after this period the results are low, *e.g.*, by 1–2% in the case of vanillin. It is therefore advisable to prepare sufficient alcoholic silver nitrate solution only for 6–8 months.

Carbon Dioxide is obtained from the usual Kipp generator, or from

a cylinder ; it is freed from hydrochloric acid vapour by passing it through sodium carbonate solution in a wash-bottle. A few pieces of string are inserted, to a distance of 10 cm., into the rubber tubing (bore, 4–6 mm.) connecting the wash-bottle with the rest of the apparatus, and with the aid of a pinchcock it is possible to obtain a very accurate adjustment of the gas-flow.

Phenol. A.R. grade is used.

Acetic Anhydride.

Nitric Acid (sp. gr., 1·4) free from halogens (see p. 89).

Tinfoil. The thickness should be such that a disc 1·5 cm. in diameter weighs not more than 12 mgm.

Cadmium Sulphate Solution, 5%.

Sodium Thiosulphate Solution, 5%.

Procedure

The apparatus is first carefully cleaned and dried by attaching a connexion from the pump to the side-tube, and drawing about 200 ml. of tap water, followed by 100 ml. of distilled water, through the apparatus, keeping the finger on the filling tube of the washer. After it has been wiped on the outside, the apparatus is dried at 120° C. Drying may be accelerated considerably by drawing acetone through the apparatus after the distilled water. The delivery tube must be perfectly clean and fat-free in order that the precipitate may be removed quantitatively, and the dried apparatus is therefore clamped in a cork in the stand and the delivery tube immersed in warm sulphuric-chromic acid for at least 5 mins. Meanwhile the material is weighed out.

It is essential that the substance should dissolve completely and, therefore, the solubility of an unknown substance must first be estimated. Two or three crystals of it and about one-third of the volume of phenol and acetic anhydride required for the analysis (p. 151) are placed in a test-tube in a water-bath at 50°–60° C. If the crystals dissolve completely, the determination may be carried out without further preliminaries. If not, the liquid is heated to boiling over a micro-burner ; if the substance then dissolves the material weighed out into the boiling flask must, similarly, first be dissolved in boiling phenol and acetic anhydride, and the hydriodic acid added only after cooling. If the material is wholly or partially insoluble even under these conditions, its solubility in more phenol (about 8 crystals) and more acetic anhydride (2 drops) is ascertained. In such cases it is helpful first to powder the substance very finely in an agate mortar. Substances (*e.g.*, pentamethyl anisole [6]) which distil out of the reaction-mixture and solidify in the condenser are heated with the reagents in a sealed tube at 135° C. for 2 hrs. ; the tube is then cooled, opened and placed in the apparatus.

Solids. A cup is made from a circular piece of tinfoil (diameter, 16 mm.) by pressing it over the rounded end of a glass rod (diameter, 5 mm.). The cup so formed is removed by turning the rod, and is then

weighed and placed on a clean tile. The substance to be analysed is added; any residue which adheres to the outside is brushed off, and the cup is placed on the balance to ascertain if the weight is approximately correct (see below). Finally, it is compressed to a tetrahedron with three fingers of the right hand, weighed after 1 min., and then carried to the apparatus on a copper block in the desiccator. If the preliminary test has shown that the material should first be dissolved in the boiling flask, the cup is very slightly compressed, so that it may be dropped into the dried flask through the side-tube, which is held vertically. If any sticks to the walls it is rinsed down with a little acetic anhydride.

Chinoy [7] describes a hollow glass "spoon," which contains the sample in the foil and is inserted vertically into the boiling flask, the exit tube of which leaves obliquely from the side; this tube also serves as the gas inlet, and so avoids the difficulty of the foil cup sticking during the insertion.

Liquids. For liquids which do not volatilise undecomposed into the cooler part of the apparatus, the micro-weighing bottle is used (Fig. 54). A glass thread is used for transferring oils; other liquids are sucked into a fine capillary, and a drop is allowed to fall into the micro-weighing bottle, and the stopper inserted. A small cup of tinfoil (weight, about 12 mgm.) must still be used for transferring the material to the flask. Hydriodic acid, which is otherwise inclined to bump, boils perfectly quietly in presence of the separated stannous iodide, and the addition of fragments of porcelain etc. is unnecessary. It is, however, important that the tinfoil never exceeds 20 mgm. in weight, because it would then reduce the concentration of the hydriodic acid so much that low methoxyl values would result. This obviously applies equally to the tinfoil cups used for solids.

The empty apparatus is removed from the clamp, and the delivery tube is rinsed down inside and out, first with distilled water and then with alcohol. For this a drop of distilled water is introduced into the upper opening with a wash-bottle or glass rod, and the cork is then inserted immediately in order to form the water-seal already mentioned on p. 148. The apparatus is then clamped loosely so that the boiling-flask is about 20 mm. above the micro-burner. The washer is charged through the filling-tube with about 0·5 ml. of 5% cadmium sulphate and 0·5 ml. of 5% sodium thiosulphate solutions, and the tube is closed with a small cork.

After the receiver has been washed successively with water, distilled water, and alcohol, it is filled up to the 2-ml. mark with alcoholic silver nitrate solution and the delivery tube is introduced by turning the apparatus outwards about the tube placed horizontally in the cork. The receiver is so placed (if necessary on a support) that the delivery tube ends at 1–2 mm. above the bottom. The ascending bubbles are flattened (or even burst) and rise slowly in the solution, and complete absorption of the alkyl iodide is ensured.

The weighed material, in tinfoil, is transferred by platinum-tipped forceps to the side-arm of the flask, and allowed to slide down it. To it are then added about 5 crystals of phenol, 5 drops of acetic anhydride and 2 ml. of hydriodic acid. If the preliminary test has shown that it is necessary, the flask is carefully warmed to boiling over the small flame of a micro-burner, before adding the hydriodic acid. To prevent superheating, the ascending tube is tapped with a pencil or wooden rod ; bumping at the beginning of boiling is thus avoided. When the material has completely dissolved the solution is cooled well and the acid added. Immediately afterwards the small sealed tube is pushed with rubber tubing into the side-tube. With the screw pinchcock closed, the cock of the Kipp apparatus is next opened, and the speed of the bubbles is so adjusted by the pinchcock that there are never more than two bubbles rising at the same time in the alcoholic silver nitrate solution.

The very small non-luminous flame of a micro-burner, with chimney, is now placed at about 15 mm. below the boiling-flask to accelerate the gas-current ; the pinchcock must, however, not be altered, as after boiling commences the ascending bubbles resume their original rate. After about 3 mins. the first indications of a precipitate at the lower end of the delivery tube are observed ; it is at first coarsely flocculent, gradually becomes crystalline, and does not usually show any apparent increase after 8–10 mins. In spite of this, it is better to allow the liquid to boil for about 20 mins., so that the last traces of methyl iodide pass over into the receiver.

The operation is concluded by removing the burner, raising the apparatus with the clamp so that the end of the delivery tube only reaches up to about one-third of the wider portion of the receiver, removing the cork from the water-seal, and rinsing the delivery tube from above, inside and out, with water acidified with nitric acid, and finally with alcohol. Particles of silver iodide still adhering to the delivery tube are rinsed in with these reagents, used alternately. Any particle obstinately retained at any point can be removed with a feather (p. 102). The contents of the receiver are diluted by the washings so that their final level is at about the middle of the widened portion. After adding 5 drops of concentrated nitric acid (free from halogen), the receiver is placed in a gently boiling water-bath until the contents begin to boil, and the silver iodide collects into a ball; it sinks after about 2 mins. The use of more or of too little nitric acid leads to high results. In the latter case especially, the silver halide double compound, $AgI,AgNO_3$, is not quantitatively decomposed in 2 mins.

After cooling under the tap, the separated silver iodide is aspirated into a filter-tube as described on p. 92, and weighed.

Calculation

1 mgm. of silver iodide corresponds with 0·1321 mgm. of OCH_3.

1 mgm. of silver iodide corresponds with 0·1918 mgm. of OC_2H_5.

log (% alkoxyl) = log (wt. AgI) + log (factor) + 2 − long (wt. substance).

$$\log 0{\cdot}1321 = \bar{1}{\cdot}12906.$$
$$\log 0{\cdot}1918 = \bar{1}{\cdot}28287.$$

Example :

Vanillin ($C_8H_8O_3$) : mol. wt., 152·1.
3·750 mgm. gave 5·78 mgm. AgI = 20·37% OCH_3.
Theory = 20·40% OCH_3.

Notes. The method fails with substances containing sulphur, because the cadmium sulphate solution in the washer does not retain hydrogen sulphide quantitatively ; even with a 10% cadmium sulphate solution, alkyl groups attached to sulphur cannot be determined. Besides methoxyl and ethoxyl groups, other lower alkyl groups bound to oxygen also yield silver iodide. Glycerol readily forms *iso*propyl iodide, and can thus be determined quantitatively. The method is also excellent for the detection and determination of alcohol of crystallisation.

Volumetric Modification

In addition to the hydriodic acid, phenol and sodium thiosulphate solution referred to on pp. 148 and 149, the following reagents are required :

Sodium Acetate in Glacial Acetic Acid (10%).
Bromine (free from iodine).
Formic Acid (80–100%).
Sodium Acetate Solution (20%).
Potassium Iodide Solution (10%).
Sodium Thiosulphate Solution. 0·02 *N*, see p. 94.
Starch Solution. See p. 173.

Procedure

The analysis is carried out in Pregl's apparatus for the determination of methoxyl (Fig. 69). The delivery tube of the apparatus is not washed with alcohol, but only with twice-distilled water, the water seal is formed and the washer is charged with the sodium thiosulphate solution. In the receiver, which has been washed with distilled water, 2 ml. of the 10% sodium acetate solution are placed, and 5 drops of bromine are added. In order to prevent the bromine vapour from entering the laboratory, a piece of cotton-wool slightly moistened with formic acid is placed in the mouth of the receiver. The filling and heating of the boiling-flask are carried out exactly as for the gravimetric method.

After boiling for 30 mins., the reaction is complete. The delivery tube is rinsed well, inside and out, with distilled water, and the inside of the receiver is rinsed quantitatively into a 100-ml. conical flask, with a ground-in stopper, in which 5 ml. of the 20% sodium acetate solution have previously been placed ; 2 drops for formic acid are then

allowed to flow along the wall, the flask is shaken and formic acid is added until the solution is colourless. After the addition of 4–6 drops of formic acid there is usually no bromine to be detected even by smell (*cf.* p. 95).

Two millilitres of the 10% potassium iodide solution are now added, the solution is acidified with 5 ml. of 2 *N* sulphuric acid and the liberated iodine is titrated after 2 mins., as described on p. 95.

Calculation

One millilitre of 0·02 *N* sodium thiosulphate corresponds with 0·6204 mgm. of OCH_3; and with 0·9008 mgm. of OC_2H_5.

$$\log (\% \text{ alkoxyl}) = \log (\text{ml. } 0{\cdot}02\, N \text{ sodium thiosulphate}) + \log (\text{factor}) + 2 - \log (\text{mgm. substance}).$$

Factor for OCH_3 = 0·10341; log factor = $\bar{1}{\cdot}01458$.

Factor for OC_2H_5 = 0·15013; log factor = $\bar{1}{\cdot}17647$.

Notes

For multiple determinations the method is quicker than the gravimetric procedure, and with reagents which in the blank test consume not more than 0·2 ml. of sodium thiosulphate solution, it gives accurate results. With vanillin the results are about 0·1–0·2% low. If substances are to be tested for traces of alcohol of crystallisation the gravimetric method is preferable, because the very small amounts of silver iodide involved can be determined.

In order that no hydriodic acid may be consumed unnecessarily, many workers advise placing the material in the boiling-flask in small glass cups, and adding red phosphorus to prevent bumping. If the substance is weighed into tinfoil cups and two analyses are to be made with one filling of the boiling-flask, then another 0·5 ml. of hydriodic acid must be added to the second sample. With natural products of unknown composition as well as with substances having resistant alkoxyl groups, it is necessary to use a considerable excess of hydriodic acid; it is, therefore, advisable to use fresh hydriodic acid for each determination.

A modified apparatus and technique for the determination of alkoxyl groups in cellulose ethers is due to Samsel and McHard [8]; important features of the method are the use of 56·5–57·0% hydriodic acid and the elimination, so far as possible, of the use of solvents. The sample is weighed out in a gelatin capsule. Glycerol in fats and phosphatides is determined by Blix [9] by a modified volumetric method, in which a solution of the sample in pure benzene is evaporated at a low temperature in presence of hydriodic acid and red phosphorus in the distillation flask. The washer contains sodium thiosulphate solution, and the receiver a solution containing sodium acetate, acetic acid and bromine; nitrogen is used to carry over the *iso*propyl iodide from the flask, which is heated in a glycerin bath at 120°–125° C. Modifications

to the washer and other parts of the apparatus, to facilitate cleaning, are also described. Ingram [15] describes the use of mercuric oxycyanide for this titration. Reference should also be made to the work of Elek,[10] which contains a general discussion of the various methods and conditions in common use. According to Elek,[10] the error of the method is $\pm$ 1%.

Determination of Methoxyl and Ethoxyl Groups in Presence of one another

The above methods give no information whether the alkyl iodide has been formed from a methoxyl or an ethoxyl group. Methoxyl and ethoxyl groups may, however, be separated by the method of Küster and Maag [11] which is based on that of Willstätter and Utzinger.[12] The alkyl iodide is collected in an alcoholic solution of trimethylamine; tetramethyl ammonium iodide and trimethylethyl ammonium iodide being formed, respectively. Since the former is almost insoluble in absolute alcohol and the latter is readily soluble, the salts may easily be separated. After the addition of dilute nitric acid and silver nitrate, the iodine is precipitated as silver iodide and weighed.

Apparatus. To ensure complete absorption in the trimethylamine, Pregl's apparatus (p. 146) must be modified. To obtain the smallest possible bubbles of gas, the gas inlet tubes are constricted to 0·5 mm. at the tips. The apparatus has two receivers. The first is connected with the washer by a ground-in joint, and consists of a small test-tube (diameter, 11 mm.; height, 65 mm.). It contains a spiral, with inlet and delivery tubes, on the principle of an extraction apparatus. The spiral has five coils with two depressions, one at the top and one at the bottom, which obstruct the bubbles for some time. The second receiver is connected with the first by a ground-in joint, and it is a test-tube (bore, 3; height, 90 mm.) the inlet tube of which is so ground that the escaping bubbles are flattened.

Procedure. Less than 5 mgm. of the sample is treated as described on p. 149. The first receiver is charged with 3 ml. of a mixture of 5 ml. of a 10% solution of trimethylamine and 12 ml. of absolute alcohol; 1 ml. of this solution is then placed in the second receiver. The apparatus is now slowly filled with carbon dioxide, the delivery of which is then stopped, and the boiling-flask is slowly heated to 140° C. in a bath of sulphuric acid so that 1, or at most 2 bubbles per sec. rise in the receiver; this rate is maintained throughout the analysis. If no more bubbles rise in the receiver after warming, the pinchcock is carefully opened and carbon dioxide is passed through the apparatus at the rate of 1 bubble per sec.

After 30 mins. the flame is turned out, and the receiver removed and kept stoppered for a day to enable the tetramethyl ammonium iodide to crystallise. For the separation of the alkyl ammonium iodide salt the spiral is removed from the receiver, placed in a small beaker, and rinsed with absolute alcohol. After the delivery tube has been

similarly rinsed, the contents of both receivers are transferred to a beaker by repeatedly rinsing with alcohol ; the solution in the beaker is finally evaporated to dryness on a water-bath and allowed to cool in a desiccator. The trimethylethyl ammonium iodide is leached from the residue with 3–4 ml. of absolute alcohol and filtered through a filter moistened with such alcohol, the last traces of the iodide being dissolved by washing the precipitate thrice more with absolute alcohol. To the alcoholic filtrate are added two to three times its volume of water, 2–4 drops of nitric acid (free from halogen), and 3 ml. of 1% silver nitrate solution, and the silver iodide is precipitated on the boiling water-bath, collected in the small filter-tube (p. 88), dried and weighed.

For the determination of the methyl group, the tetramethyl ammonium iodide is dissolved from the filter with hot water, the spiral and inlet tube are rinsed, and all the washings are collected in the beaker used for the evaporation. The precipitation and weighing of the silver iodide follow, as above. The error is $\pm$ 1%.

The apparatus used for the determination of methoxyl or methylimino groups (p. 146) has been used successfully for the detection of alkyl radicals, the precipitate being collected in a small centrifuge tube and identified by determinations of the carbon and hydrogen contents. Methoxyl and ethoxyl groups may also often be distinguished by the method of Kuhn and Roth for the determination of methyl groups attached to carbon (p. 167). If the sample is oxidised with chromic acid, no acetic acid is found when only methoxyl is present. In presence of an ethoxyl group, however, an amount of acetic acid equivalent to the silver iodide weighed is formed. If (*e.g.*) the substance contains one group of each alkoxyl per molecule, 1 molecule of acetic acid is obtained from the ethoxyl group only. This method is modified if other methyl groups attached to carbon, acetyl, or benzoyl groups are present (*cf.* p. 167).

A further method is due to Friedrich.[13] The oxygen of the alkoxyl groups is calculated from the iodine determined by Pregl's method ; or, as iodate, by the method of Vieböck and Brecher (p. 146). The alkyl iodide formed in the same way from a second sample is not led into the receiver, but directly into a micro-combustion tube (having red-hot platinum contacts) for the determination of carbon and hydrogen, the alkyl iodide being burnt to carbon dioxide and water (p. 36). From the increase in weight of the soda-lime the carbon content of the alkoxyl groups may be calculated. From these results the proportion of oxygen atoms to carbon atoms in the alkoxyl groups may be calculated. If the ratio O : C = 1 : 1, a methoxyl group is present ; if 1 : 2, an ethoxyl group ; and if 2 : 3, one methoxyl group is present to one ethoxyl group. Furter[14] identifies alkyl groups linked to oxygen or to nitrogen atoms by forming the corresponding dinitrobenzoic acid esters and α-naphthylamine compounds, and determining their melting-points.

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DETERMINATION OF METHYL- AND ETHYL-IMINO GROUPS

Pregl used the principle of the Herzig-Meyer macro-method. The alkyl compound was converted by means of hydriodic acid into the quaternary ammonium salt, which was decomposed at 300°–360° C. with separation of the alkyl iodide, which was determined gravimetrically as silver iodide. The apparatus shown in Fig. 70 was devised for the purpose in 1915.

Since then, this method has been very thoroughly studied by various workers, notably Edlbacher,[1] Friedrich,[2] Slotta and Haberland[3]; Friedrich's apparatus (Fig. 71), in which the flow of gas is not hampered by the condensed hydriodic acid is, in particular, a notable advance in technique. Further, Edlbacher found that the washer (which originally contained a suspension of red phosphorus) did not absorb completely the carbon dioxide and the vapours of hydriodic acid and hydrogen sulphide which pass through it ; precipitation of silver sulphide in the receiver may easily occur, particularly during a sudden increase in the speed of the gas. For this reason Edlbacher used two washers filled with a 5% solution of cadmium sulphate. Friedrich prefers a mixture of 5% cadmium sulphate and 5% sodium thiosulphate solutions for gravimetric work.

The volumetric method of Vieböck and Brecher[4] (p. 160) is, however, much simpler because hydrogen sulphide does not interfere and therefore, commercial hydriodic acid (as in the Zeisel methoxyl determination) can be used so long as its concentration is not less than corresponds with sp. gr. 1·7. It is, however, advisable to retain the thiosulphate solution in the receiver, and Slotta and Haberland use a 5% solution to which a little sodium carbonate is added.

Decomposition products of lactoflavin and synthetic flavins give low or fluctuating results,[5] but if these substances are first warmed or gently boiled with an excess of phenol and acetic anhydride until a clear solution results, and ammonium iodide, gold chloride, and hydriodic acid are added after cooling, the results are excellent. The prevalent view that methyl-imino groups which are difficult to split

off owing to their particular linkages are the source of low results is therefore, open to question, because such substances (*e.g.*, ξ-trimethyl pentadecabetaine [6]) may be dealt with by the above modification. For safety's sake, therefore, it is advisable to dissolve every substance (except, of course, those which are readily soluble) before the analysis, a precaution which takes only 5–7 mins. It is interesting to note that work at the Kaiser-Wilhelm Institut showed that the consumption of thiosulphate by thiazole picrate corresponds with exactly one CH_3 group per molecule; and by 2-amino thiazole, with one CH_3 for 4 molecules of substance. With α-μ- and β-μ-dimethyl thiazoles no thiosulphate was used up.

Apparatus

Pregl and Lieb (Fig. 70). This consists of the small flask *SK*, of about 20 mm. diameter, and the slightly inclined distilling tube *SR* (length, 150–160 mm.; external diameter, 6–7 mm.); thicker tubes necessitate several distillations. The other inclined tube (*A*) is at least 100–140 mm. long (*cf.* Fig. 69), and the sealed tube is inserted in it as in the methoxyl apparatus. The distilling tube (*SR*) is bent slightly downwards for a length of 60 mm., and it then descends vertically into the receiver *V*. The almost horizontal portion is surrounded by a split cork, so that the whole apparatus may be supported by a clamp at this place.

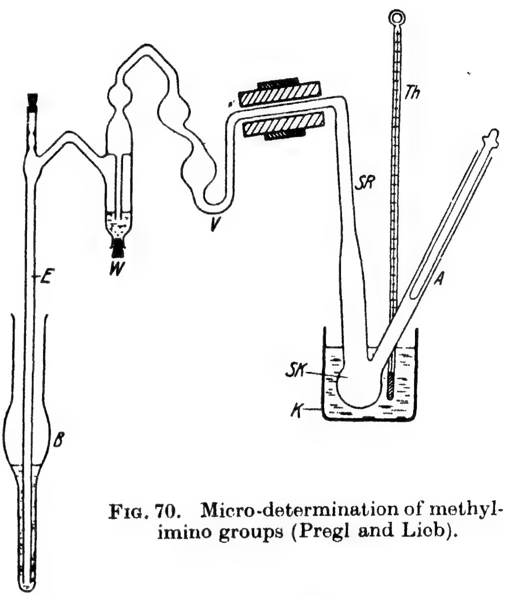

FIG. 70. Micro-determination of methyl-imino groups (Pregl and Lieb).

The receiver for the distilled hydriodic acid (*V*) consists of two connected bulbs, so arranged as to avoid spirting. The washer (*W*), which follows them, differs from the washer of the methoxyl apparatus in that its upper portion is so large that, on sucking back the hydriodic acid before beginning a fresh distillation, the whole of the contents of the washer are retained in it.

The gas-delivery tube *E*, which follows the washer, is exactly the same as in the apparatus for the determination of methoxyl, and the same form of widened test-tube (*B*) is used for holding the alcoholic silver nitrate solution. The flask is heated in a copper or iron can *K* (diameter, 50; height, 50 mm.) which is filled with powdered

copper oxide residues from old combustion tubes. The temperature can be read accurately on a thermometer (*Th*) dipping into the copper oxide. A flask made of Jena-type glass can be used for 50–80 analyses.

Friedrich (Fig. 71). In contrast with the above apparatus the stream of gas does not pass through the condensed hydriodic acid. No hot vapours, therefore, enter the washer, and once the rate of bubbling has been established it remains constant; at 360° C. the apparatus may be left to itself.

At the beginning of the determination the gas current can pass *via* *AA'*, or *BB'*, the cock in *BB'* being open. Later, when the hydriodic

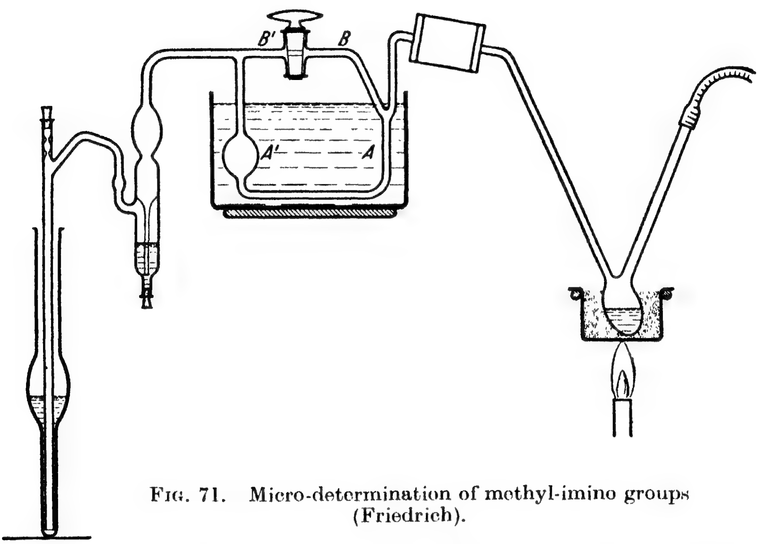

FIG. 71. Micro-determination of methyl-imino groups (Friedrich).

acid has condensed and collected in *A'*, the route *BB'* only is available. Apart from its normal bore the cock has a vertical groove which ends before the middle of the bore. If the cock is turned clockwise through 90° from the position shown in Fig. 71, the passage of gas is cut off and at the same time *B'* is connected with the outside air. The whole apparatus is made of Jena-type glass; quartz is unnecessary.

Reagents.

In addition to those enumerated for the gravimetric determination of methoxyl (p. 148), *ammonium iodide* and a 5% aqueous solution of *gold chloride* are required.

Procedures

Pregl and Lieb. The apparatus is cleaned, and provided with a water-seal exactly as described for the methoxyl determination. With the long-handled weighing-tube (p. 73) the material to be analysed is passed into the boiling-flask through the tube *A* (which is held horizontally). The washer is about one-third filled with a mixture of equal volumes of sodium thiosulphate and cadmium sulphate

solutions, and closed. About 50 mgm. of phenol and 3–5 drops of acetic anhydride are added to the sample through the side-tube, and the substance is dissolved by warming carefully over a micro-burner. If the amount of solvent present is insufficient, more phenol is added.

The flask is cooled again, and the delivery tube is dipped into the receiver, which holds exactly 2 ml. of alcoholic silver nitrate solution. To the dissolved substance are added about 30 mgm. of ammonium iodide, a small ball of tinfoil, 1 drop of gold chloride solution, and, finally, 2 ml. of hydriodic acid. The Kipp apparatus is connected to the side-tube, and the gas current is regulated exactly as in the methoxyl determination so that not more than 2 bubbles rise in the receiver at the same time. The boiling flask is heated in the copper or iron pan, and a new receiver is inserted after 45 mins., without interrupting the heating. The temperature is then raised to 200° C. in 20–25 mins. If the heating is too rapid vapours of hydrogen sulphide and hydriodic acid enter the washer with the carbon dioxide, and are not completely retained in it. Finally, the copper oxide bath is heated at 350°–360° C. for at least 30 mins.; and the experiment is completed by extinguishing the flame and maintaining a stream of carbon dioxide while the gas inlet-tube is rinsed into the lowered receiver, first with water acidified with nitric acid, and finally with alcohol. The precipitate is treated as in the methoxyl determination, and the receiver is placed (protected from halogen vapours) in a test-tube stand.

Before sucking back the hydriodic acid for the second distillation, it is advisable to empty the washer and add fresh solutions. When the temperature of the copper oxide bath is below 100° C., the hydriodic acid is carefully sucked back into the flask in such a manner that no washing liquid is drawn back into the receiver, *e.g.*, by means of a rubber tube slipped over the side-tube of the boiling-flask. The distillations are repeated until the separation of the silver iodide double compound is only just perceptible, and not more than 0·5% is formed.

Friedrich. Up to the heating stage the procedure is similar to that described above, the cock being set so that *BB′* is open. As soon as heating is started, the U-shaped part of the apparatus is immersed in a glass dish (Fig. 71), which is kept full of hot water throughout the determination, so as to prevent condensation in this part of the apparatus.

After the determination, the stream of carbon dioxide is stopped with the pinchcock, and the glass cock is turned so that communication is established between *B′* and the outer air; the contents of the flask are cooled and the distilled hydriodic acid is thus aspirated back into the flask, without any danger of also aspirating back the contents of the receiver or the washer. If the boiling-flask is already too cold, the hydriodic acid is sucked back by means of tubing. After suction,

a few drops of hydriodic acid still remain in AA'; in order to bring this residue into the flask the delivery tube is rinsed out, while some hydriodic acid is allowed to flow in from a pipette through the groove and is then slowly aspirated through as before. Renewal of the washing liquid is unnecessary.

Volumetric Determination of Alkylimino and Alkyl Groups attached to Sulphur [4]

The boiling-flask (Figs. 70 and 71) is charged as for the gravimetric determination. As in the volumetric determination of methoxyl, 5% sodium thiosulphate solution is placed in the washer, to which 0·5% of sodium carbonate is added as suggested by Slotta and Haberland.[3] The receiver is charged as described on p. 158, and the iodate is titrated after each distillation as described on p. 153. When only 1 or 2 drops of thiosulphate are required for the titration the analysis is taken as complete. The volumetric method is preferred to the gravimetric method, because the latter sometimes fails with methyl groups attached to sulphur; even with cadmium sulphate solution and a slow passage of carbon dioxide, precipitation of silver sulphide occurs.

Calculations

(*a*) **Gravimetric :**

log (% alkyl) = log (wt. of AgI) + log (factor) + 2 − log (mgm. substance).

For CH_3, factor = 0·06398 ; log factor = $\bar{2}$·80604.

For C_2H_5, factor = 0·12380 ; log factor = $\bar{1}$·09273.

Example :

Atropine, $C_{17}H_{23}NO_3$; mol. wt., 289·18, 5·740 mgm. taken.
1st distillation, 4·16 mgm. AgI.
2nd distillation, 0·54 mgm. AgI.

Total,	4·70 mgm. AgI	= 5·24% CH_3.
Theory for	1 CH_3	= 5·20% CH_3.

(*b*) **Volumetric :**

log (% alkyl) = log (ml. 0·02 *N* $Na_2S_2O_3$) + log (factor) + 2 − log (mgm. substance).

For CH_3, factor = 0·3005 ; log factor = 1·47780.

For C_2H_5, factor = 0·5808 ; log factor = $\bar{1}$·76403.

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DETERMINATION OF ACETYL AND BENZOYL GROUPS

History and Principle of the Method

Prior to about 1933 most of the existing methods [1-3] gave accurate results in expert hands; they are, however, not easy to manipulate, especially if they are carried out only occasionally or by students. The following method due to Kuhn and Roth,[4] has made possible the simple determination of acetyl and benzoyl groups by acid, alkaline, and alcoholic saponification with a degree of accuracy of $\pm$ 0·3—0·5%. It was first used for the determination of methyl groups attached to carbon by oxidation with chromic acid. After the substance had been saponified, the acetic or benzoic acid was distilled off at atmospheric pressure from solution in sulphuric acid, through a quartz condenser, and titrated.

According to Pregl [2] distillations made through glass or quartz condensers give incorrect results, but it appears that a quartz condenser which is completely transparent is satisfactory. Further, in order to avoid the formation of sulphur dioxide, phosphoric acid was used instead of sulphuric acid for the saponification and distillation. Unfortunately all such experiments failed, because phosphoric acid was always to be found in the distillate, *e.g.*, to the extent of 1·6 mgm. per 100 ml.; the acetic acid, therefore, could not be driven off from the primary sodium phosphate solution. However, it was then found iodimetrically that the sulphur dioxide was completely expelled from the distillate after boiling for 3–4 secs. with sulphuric acid, whilst Friedrich and Rapoport [5] in exhaustive investigations into the removal of carbon dioxide from solutions of acetic acid without loss of acetic acid, had already found 7–8 secs. to be an optimum boiling time; this time is ample for the removal of all sulphur dioxide. The expulsion of both carbon dioxide and sulphur dioxide is thus ensured.

Many of the above difficulties are overcome by hydrolysis with an alcoholic solution of acid. The resulting ethyl acetate is hydrolysed by an excess of alkali, which is then back-titrated. The method has been adapted by Clark [6] to the semi-micro scale. It is essential that the solubility of the substance to be analysed and the strength of the linkage of the acetyl or benzoyl group should be taken into account. Particular attention must be paid to the possibility that, besides acetic or benzoic acid, other volatile acid decomposition products may be formed.

The solubility of a very small sample of the substance in four saponification agents is first tested in a test-tube. If the acyl group is combined with oxygen it is usually saponified on the boiling water-bath for 20 mins. with sulphuric acid or *p*-toluene sulphonic acid; or for 5 mins. with a solution of sodium hydroxide in water or methyl alcohol. There are, however, compounds in which the group is attached to oxygen which must be saponified with sodium hydroxide in methyl alcohol for 2·5 hrs., *e.g.*, the methyl ester of triacetylcholic

acid. Acetylated catechin, anthocyanidin, etc., yield high values on alkaline saponification; and acetylsalicylic acid gives exactly twice the theoretical result because, besides acetic acid, the salicylic acid is also titrated. In such cases if other methyl groups attached to carbon are absent, it is essential that the chromic acid method described on pp. 167 *et seq.* should be used; the volatile acids which occur in addition to acetic acid are destroyed by oxidation. Compounds in which acetyl or benzoyl groups are attached to nitrogen are usually saponified with alkali in methyl alcohol, to avoid a long saponification time.

It is only very rarely that the substance is insoluble in the above reagents. In such cases it is first dissolved in 1 ml. of pure pyridine (p. 138), sodium hydroxide in methyl alcohol is added as above, and the pyridine and methyl alcohol are distilled off after the saponification which follows. The pyridine which still remains in the saponification flask is fixed as sulphate after acidification, and does not affect the determination. Substances which undergo auto-oxidation, forming volatile acid decomposition products with water vapour, are preferably saponified and distilled in a current of nitrogen.

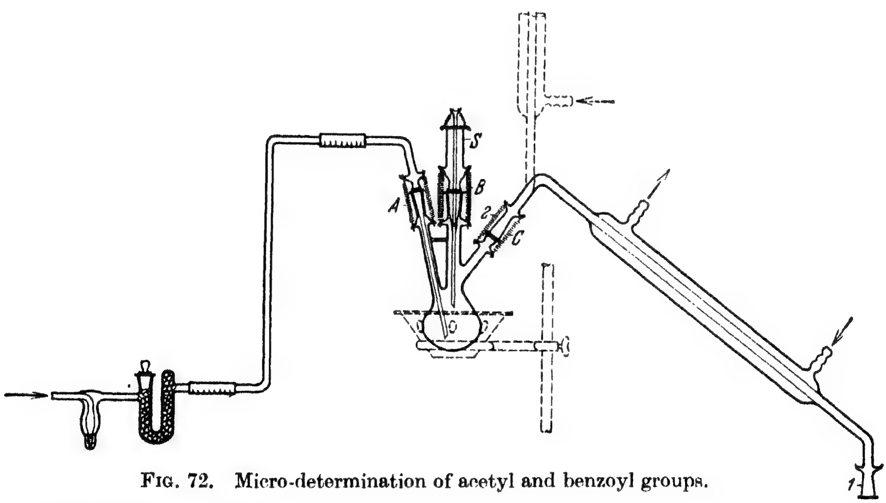

FIG. 72. Micro-determination of acetyl and benzoyl groups.

Apparatus (Fig. 72)

The Bubble-Counter with the sealed-on U-tube is similar to that used for the determination of carbon and hydrogen (p. 35). If charged with 50% sodium hydroxide solution, it serves both for the purification of the oxygen, and for measuring the speed of the gas. The U-tube is filled with soda-lime or Ascarite.

The Jena-type Glass Flask (capacity, 45 ml.) is pear-shaped and has three limbs, *A*, *B* and *C*, with ground-in joints (see Fig. 72). Through the limb *A* (length, 80 mm.; bore, 6 mm.) passes the gas inlet tube (bore, 2 mm.) which is widened to fit the joint at the top, and reaches almost to the bottom of the flask. The bubbles escaping from it prevent superheating. The limb *B* is 80 mm. long, and the hollow

joint (diameter, 10 mm.) holds the stopper with a funnel inside it, and reaching slightly below the bottom of the limb. With the glass rod *S* inserted it has a capacity of 8 ml.; marks are made at 2 and 7 ml. By raising the ground-in glass rod, water may be added without interrupting the distillation. A steel spring or a rubber band presses the glass rod back again so that no more water can flow in. To keep the apparatus firm, the limbs *A* and *B* are connected by a sealed-on glass rod. The limb *C* (length, 65 mm.; bore, 50 mm.) is inclined to the horizontal at 50°, and its ground-in end forms an absolutely air-tight joint with the quartz condenser. All the stoppers are secured with steel springs. The substance is saponified by immersing the flask in a boiling water-bath (*e.g.*, a litre beaker) so that the water reaches up to the bottom of the limbs; during the oxidation with chromic acid it is better to heat in a small Babo funnel.

The Quartz Condenser is made from completely transparent quartz and has a ground-in joint at each end, which fits exactly into the limb *C*. The total length is 36 cm.; 4·5 cm. from the joint *1* the condenser is bent at 40°. If the joint *1* is connected with *C*, the condenser acts as a reflux condenser. At a distance of 5·5 cm. from the joint 2 the condenser is bent through 90° as shown; if *2* is inserted in *C*, a distilling condenser is formed.

Reagents

Reagents marked * are necessary only if acetyl groups are to be determined as "methyl groups attached to carbon" (see p. 167).

Sodium Hydroxide Solutions. 5 *N* and *N*.

Sodium Hydroxide in Methyl Alcohol. *N*. Four grams of sodium hydroxide, in tablet form, are dissolved in a mixture of 50 ml. each of water and methyl alcohol. To remove any traces of acid, the methyl alcohol is usually first refluxed for about 15 mins. with solid potassium hydroxide, and then distilled.

Wenzel's Sulphuric Acid. To 200 ml. of water add 100 ml. of sulphuric acid, sp. gr. 1·84.

***p*-Toluene Sulphonic Acid.** A 25% solution in water.

* **Chromic Acid.** 5 *N*. A solution of 168 gm. of the anhydride (as used for the determination of carbon, see p. 62) in 1 litre of water is filtered through a suction filter having fine pores.

* **Sulphuric Acid,** sp. gr. 1·84.

* **Phosphoric Acid,** sp. gr. 1·7 (syrupy).

* **Hydrazine.** A mixture of 10 ml. of hydrazine hydrate with 10 ml. of water.

Metaphosphoric Acid. Prepared from phosphorus pentoxide and a few drops of water.

Barium Chloride. Crystalline.

Sodium Hydroxide Solution (0·01 *N*).

Hydrochloric Acid (0·01 *N*) in a micro-burette with an automatic zero-adjustment (Fig. 25).

Procedure

If the apparatus is new or has not been used for some time, it is first treated with warm sulphuric-chromic acid to remove any impurities present. All the glass parts are cleaned before every determination with distilled water, and dried in a stream of warm air, or in the drying-oven at 110° C. The condenser is cleaned similarly, but it is not dried.

Weighing. It has been found best in practice to weigh out as much substance as will require 3–6 ml. of 0·01 *N* acid, in the long-handled weighing tube (p. 73); the substance is then transferred to the flask.

Substances which are difficult to dissolve are first powdered as finely as possible in an agate mortar. Substances which have been dried quantitatively are allowed to slide, in a boat, through limb *B* by slanting this. Syrupy liquids are treated similarly. Liquids are weighed by Pirsch's method (p. 202). The capillary is placed point downwards in the saponification agent (which is already in the flask) or in the sulphuric-chromic acid solution, the condenser being in position; the capillary is broken by pressing on the handle with a glass rod. If the rod becomes moist it is rinsed with 1 ml. of water. The ground-in funnel is then quickly inserted and secured. The bubble-counter is connected with an oxygen or nitrogen gasholder by rubber tubing, and the velocity of the gas-current is adjusted by a needle-valve or a pinchcock to 50 bubbles per min.

Saponification. For acid saponification, 1 ml. of Wenzel's sulphuric acid or 1 ml. of the *p*-toluene sulphonic acid solution; for alkaline saponification, 1 ml. of 5 *N* sodium hydroxide or 4 ml. of *N* sodium hydroxide in methyl alcohol solution are used.

The flask is firmly clamped by the limb *B*, the joint of *C* is moistened with water, and the condenser is fitted tightly in the reflux position, secured with the steel springs, and clamped in the middle. The joint of the gas inlet-tube and the outer joint of the small funnel are now moistened with metaphosphoric acid, and each is held by two steel springs. Connexion with the bubble-counter is then made, the glass rod *S* is tightly fitted in, and the funnel is charged with 2 ml. of water. The substance is now saponified by heating the flask in a boiling water-bath.

Distillation and Titration. The factor for the 0·01 *N* sodium hydroxide is determined under conditions which approximate as closely as possible to those of the titration to be made later. In the 100-ml. quartz flask (see Kjeldahl determination, p. 78) 3–4 mgm. of pure oxalic acid ($2H_2O$) are dissolved in 20 ml. of water, the carbon dioxide is boiled off in 7 secs. while shaking over an open flame, and 4–5 drops of 1% phenolphthalein solution are added. The mixture is titrated rapidly with 0·01 *N* alkali to a faint pink colour; 3–6 ml. of alkali are required.

When the saponification is finished and the solution is cool, the glass rod *S* is taken out; the condenser is rinsed into the flask with

5 ml. of water, and the glass rod is replaced. The condenser is then removed, washed with 100–200 ml. of water, and replaced as a distilling condenser, all connexions being secured with the springs. If the saponification has been carried out with alkali in methyl alcohol, 5 ml. are distilled over (to remove the methyl alcohol), and the condenser is rinsed as before and replaced ; the clamp at *B* should be loose.

The acid is neutralised by : (*a*) 1 ml. of 5 *N* sodium hydroxide (saponification with sulphuric acid) ; (*b*) 0·5 ml. of *N* sodium hydroxide (saponification with *p*-toluene sulphonic acid) ; or (*c*) 1 ml. of Wenzel's sulphuric acid is placed in the funnel (hydrolysis by alkali). By carefully raising the glass rod the alkali or acid is allowed to flow from the funnel into the flask, and 2 ml. of water are used to rinse it in. Finally, for the first distillation, water is added up to the mark 7 on the funnel, and three small pieces of pumice are placed in the flask. The limb *B* is then clamped tightly, the bubble-counter is connected, and the funnel is fitted in the joint, which has been smeared with metaphosphoric acid. The subsequent distillation of the acetic or benzoic acid determines the acetyl or benzoyl groups, respectively, and also the acetic acid produced by oxidation.

The distillates are collected in a 25-ml. Jena-type glass measuring cylinder with a funnel, and in order to drive over the whole of the acids, it is advisable to warm in a small Babo funnel, so that 5–6 ml. of distillate condense in 5 mins. When the volume of liquid in the flask is 2–3 ml., water is slowly run in to mark 2, by raising the glass rod *S* ; the distillation is not interrupted. This procedure is continued until the end of the determination. After the first four 5-ml. portions have been collected, the measuring cylinder and funnel are removed, and the quartz flask is quickly slipped under the end of the condenser. The funnel is then placed in this flask, and the distillate is poured into the flask from the measuring cylinder. The measuring cylinder is replaced under the end of the condenser, without rinsing, and the contents of the flask titrated ; thus, 2–3 crystals of barium chloride are added, and the liquid is boiled (as in the determination of the factor), and titrated in presence of phenolphthalein. Subsequent distillates are titrated similarly. Turbidity after boiling (due to barium sulphate) occurs only very rarely (*e.g.*, through superheating), and the analysis is then spoiled.

If less than 4 ml. of 0·01 *N* alkali are used up in the titration of the first 20 ml., then the next 10 ml. of distillate are titrated ; if more are required, then 15 ml. of distillate are collected for the second titration. If the liquid in the distillation-flask is concentrated to 2–3 ml. before the distillation of each 5-ml. portion, then after the first two titrations approximately 1% of the acetic acid still remains (see example below). When the end-point appears with 1 drop (0·01–0·02 ml.) of standard alkali, the analysis is finished. The whole distillation requires 30–40 mins.

The acetic or benzoic acid may be determined by one titration on all the distillates together, but this is not advisable. Thus :

1. If no acetic or benzoyl group can be detected, the analysis is finished with the first titration and further distillations are avoided.

2. From the titration of the first 20 ml., the number of further distillations necessary may be estimated.

3. If, in consequence of superheating, sulphate is detected in the distillate from the third or fourth distillation, then 99·0–99·5% of the acetic or benzoic acid has already been titrated in the previous satisfactory distillates and the analysis is not spoiled.

4. The method ensures the quantitative distillation of acetic or benzoic acid.

Example :

	ml. distillate	ml. 0·01 *N* NaOH used
1st titration . . .	20	6·00
2nd ,, . . .	15	0·30
3rd ,, . . .	5	0·05
4th ,, . . .	5	0·02
Total . .	45	6·37

Calculation

log (% acetyl) = log (ml. 0·01 *N* NaOH used) + log (factor) + 2 − log (mgm. sample).

For acetyl, Factor = 0·4302 ; log Factor = $\bar{1}$·63370.

For benzoyl, Factor = 1·0504 ; log Factor = 0·02135.

Examples :

Acetyl-glycine, $C_4H_7NO_3$ (mol. wt. 117·05).
Theory, 36·75% $COCH_3$.

mgm. substance	ml. 0·01 *N* NaOH used	% $COCH_3$ found
5·604	4·74	36·57

(Saponified for 2·5 hrs. with sodium hydroxide in methyl alcohol.)

Psicain, $C_{17}H_{21}O_4N.C_4H_6O_6$ (mol. wt. 453·2).
Theory, 23·18% COC_6H_5.

mgm. substance	ml. 0·01 *N* NaOH used	% COC_6H_5 found
15·922	3·53	23·28

(Saponified for 1 hr. with 5 *N* sodium hydroxide.)

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DETERMINATION OF METHYL GROUPS ATTACHED TO CARBON

History and Principle of the Method

As early as 1888, Messinger [1] used chromic acid for the determination of carbon in organic substances by wet oxidation. Low values were obtained with fatty acids, but their complete combustion was ensured by Simon [2] by oxidation in presence of silver chromate (*cf.* p. 62).

In the method now described [3,4] the substance is refluxed with a sulphuric-chromic acid mixture under conditions such that the compounds containing methyl groups attached to carbon are decomposed with the formation of acetic acid, and acetic or benzoic acid already previously present is unaffected. Since, however, liquids and volatile products of the decomposition of solids may pass into the colder part of the apparatus before they have been quantitatively oxidised, micro-bombs have been used; it is thus possible to determine the methyl groups attached to carbon with great accuracy, even in extremely volatile substances such as ether. After reduction of the excess of chromic acid, the acetic or benzoic acid is fractionally distilled in presence of phosphoric acid, and titrated.

The amount of acetic acid formed by the oxidation of compounds containing methyl groups attached to carbon depends very largely on the other linkages of the $C—CH_3$ group (*cf.* p. 169). Ethoxyl groups give theoretical yields of acetic acid, and the method may be used for the determination of ethoxyl on the one hand and, on the other, to ascertain the sum of ethoxyl and acetyl present; thus the di-ethyl ester of tetra-acetyl mucic acid yields four acetyl and two ethoxyl groups. If methoxyl and ethoxyl are to be differentiated, the sum of the alkoxyl groups is first determined as described on p. 146, and afterwards the ethyl groups are determined as acetic acid by oxidation with chromic acid. Accurate determinations of acetyl and benzoyl groups can sometimes be made only by this method (*cf.* p. 161).

Reagents. See p. 163.

Apparatus. See p. 161.

Procedure

Solids. After the solubility in the oxidation-mixture of the very finely powdered substance has been ascertained, the sample is weighed, put into the flask and the condenser attached. Then 5 ml. of the cooled oxidation-mixture (20 ml. of 5 *N* chromic and 5 ml. of sulphuric acid, sp. gr. 1·84) are added; some anthocyanidins must first be dissolved in sulphuric acid, chromic acid being added drop by drop, with cooling. The inlet tube and funnel are connected, and the mixture is boiled for 1·5 hrs. under a reflux condenser; the flask may be heated in a small Babo funnel.

After the mixture has cooled, the glass rod *S* (Fig. 72) is removed,

the condenser is rinsed down with 5–7 ml. of water, the oxygen gas-holder is disconnected, and the condenser, the inlet tube (which has been rinsed), and the small funnel are removed.

Liquids (and solids which are volatile, or which form volatile decomposition products which escape from the oxidation mixture) are oxidised in a small pressure-tube, 30 cm. long (*cf.* p. 98). Solids are transferred to the pressure-tube by means of the long-handled weighing tube (p. 73), and the residues adhering to the wall are rinsed in with 5 ml. of the sulphuric-chromic acid mixture. Liquids with low vapour pressures are introduced in micro-weighing bottles (*cf.* p. 97); very volatile liquids (*e.g.*, ether) are weighed by Pirsch's method (p. 202), the sulphuric-chromic acid mixture being prepared and cooled in the pressure tube. If the mixture is warmer than the capillary the liquid may volatilise before the tube is sealed, and the capillary must be pushed point downwards into the oxidation-mixture and the tube cooled and sealed quickly (*cf.* p. 98).

The micro-pressure tube is heated (as described on p. 97) at 120° C. for 1·5 hrs., and cooled. It is then opened (*cf.* p. 98), the tip is broken off and rinsed into the oxidation-flask with 1–2 ml. of distilled water, and the capillary in the pressure-tube is crushed with a glass rod. The mixture and rod, with the fragments of glass, are rinsed quantitatively into the oxidation-flask with 6–8 ml. of distilled water.

To reduce the excess of chromic acid, dilute hydrazine solution is added drop by drop, with cooling, until the first tinge of green is observed; an end-point of pure green must be avoided. After further cooling, the mixture is neutralised with 6 ml. of 5 N sodium hydroxide and then acidified with 1 ml. of phosphoric acid (sp. gr., 1·7); three pieces of pumice are placed in the flask, and the distillations and titrations proceed as in the acetyl determination (p. 161).

Calculation

1 ml. of 0·01 N NaOH corresponds with 0·15023 mgm. CH_3 or 0·60031 mgm. $CH_3.COOH$.

$$\text{For } CH_3\text{, log factor} = \bar{1}\cdot17676.$$

$$\text{For } CH_3.COOH\text{, log factor} = \bar{1}\cdot77838.$$

$$\log(\%\ CH_3) = \log(\text{ml. } 0\cdot01\ N\ \text{NaOH}) + \log(\text{factor}) + 2 - \log(\text{mgm. sample}).$$

Examples:

Acetyl-salicylic acid, $C_9H_8O_4$. Mol. wt., 180·03.

mgm. substance	ml. 0·01 N NaOH	% CH_3 (to C)
13·718	7·66	8·38 Found.
		8·34 Theory.

Time of oxidation, 75 mins.

Bixin, $C_{25}H_{30}O_4$. Mol. wt. 394·23.

mgm. substance	ml. 0·01 N NaOH	% CH_3 (to C)
9·275	9·34	15·13 Found.
		15·24 Theory.

Time of oxidation, 60 mins.

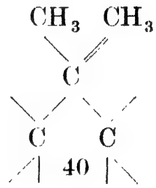
Ether, $C_4H_{10}O$. Mol. wt., 74·07.

mgm. substance.	ml. 0·01 *N* NaOH.	% CH_3 (to C).
2·513	6·78	40·53 Found.
		40·54 Theory.

Time of oxidation at 120° C., in pressure tube, 90 mins.

YIELDS OF ACETIC ACID, AS PERCENTAGES OF THEORETICAL

$C_2H_5.OH$	$C_2H_5.O.C_2H_5$	$C_2H_5O.CO.R$
100	100	95–100
$CH_3.CO.OR$	$CH_3.CO.CH_2.R$	$CH_3.CHOH.CHOH.R$
100	85	95
$CH_3.CH=CH.R$	$=CH.C(CH_3)=CH—$	
85	90	



Compound	Molecular fraction oxidised	Acetic acid as percentage of theory
Acetophenone	0·1	10
m-Xylene	0·24	12
1, 3-Dimethyl-2-hydroxybenzene	1·1	55
Thymol	1·4	70
o-Methylamine	0·7	
1-Hydroxy-4-methyl-2-benzoic acid	0·75	
p-Toluidine	0·60	
m-Xylidine	1·2	60
6, 8, 9-Trimethyl-*iso*alloxazine (a *m*-xylene derivative)	1·40	70
6, 7, 9-Trimethyl-*iso*alloxazine	0·9	45
N-Ethylaniline	0·9	

Assuming "additivity" for α-ionone, according to the above data there are to be expected 0·4 + 0·85 + 0·85 = 2·1 molecules of acetic acid per molecule. There were actually found 2·0 molecules.

It should here be emphasised that higher fatty acids are not always decomposed uniformly to acetic acid. In cases of doubt, therefore, it is advisable to distil over the acid produced from a larger sample and to treat it with *p*-bromophenacylbromide so that the acid can be identified from the melting-point of the corresponding *p*-bromophenacylester [5, 6]; the melting point of sodium acetate [7] (324° C.) provides a further check.

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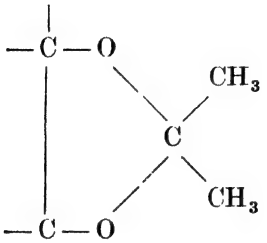
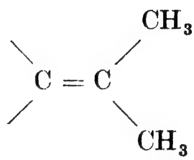
DETERMINATION OF *ISO*PROPYLIDENE GROUPS

Theory of the Method

*Iso*propylidene groups attached to oxygen (as in acetonic compounds of sugar, ketonic acids, etc., see formula I) may be quantitatively decomposed by dilute acids and determined by titration of the acetone as iodoform.[1-3] *Iso*propylidene groups attached to carbon (formula II) are split off as acetone by means of ozone. Aldehydes which react similarly with hypoiodite must first be decomposed by permanganate [4,5] before distilling over the acetone.



(I)



(II)

The reactions are :

$$3I_2 + 6KOH \rightarrow 3KI + 3KIO + 3H_2O,$$
$$CH_3.CO.CH_3 + 3KIO \rightarrow CHI_3 + CH_3.COOK + 2KOH.$$

The published work [2,6,7] on the factors affecting these reactions is contradictory. Too much iodine is consumed if impure reagents are used ; he purity of the water is very important. Acidification of the alkali by sulphuric acid before the titration, and the use of only a 50–100% excess of iodine should be ensured. The titration error due to dilution can be neglected under the conditions described. Experiments showed that under these conditions the acetone formed is stable to ozone and to permanganate acidified with acetic acid. Thus even with small amounts (2 or 3 mgm.) of acetone, the absolute error is less than $\pm$ 0·5%.

Whilst *iso*propylidene groups attached to oxygen (I) may be determined with great accuracy, the theoretical amount of acetone is seldom obtained after ozonisation of compounds in which these groups are attached to carbon (II) ; thus all terpenes which are credited with "acetone groups," have acetone values below those expected. Since the deficit is frequently accounted for by formic acid and formaldehyde, Grignard and his co-workers [8] concluded that equilibrium mixtures of compounds containing CH_2 groups

$$CH_2 : C(CH_3)CH_2 \qquad (a\text{-form}), \qquad (III)$$

and compounds containing the *iso*propylidene radical

$$(CH_3)_2C_2 : CH \qquad (\beta\text{-form}) \qquad (IV)$$

are present. Simonsen, an authority on terpene chemistry, considers that even in crystalline compounds (which to all appearance are homogeneous) equal amounts of the α- and β-forms are present. The absorption spectrum of synthetic dehydrogeranic acid [9, 10] (m.p. 137° C.) (V) approximates very closely to that of octatrienoic acid (VI), so that apparently only the *iso*propylidene, or β-form is present.

$$(H_3C)_2C = CH—CH = CH—C(CH_3) = CH—COOH \qquad (V)$$

$$H_3C—CH = CH—CH = CH—CH = CH—COOH \qquad (VI)$$

With (V) the disagreement between the results obtained by ozonisation and from the absorption spectrum suggests that, on decomposition by oxidation with ozone or permanganate, β-forms may partly yield decomposition-products of the α-type. The incomplete formation of acetone on the decomposition of *iso*propylidene compounds with ozone is probably due to reactions which are closely connected with the known rearrangement of acetone peroxide to form methyl acetate. With natural products, therefore, numerous comparisons of the acetone obtained with that given by compounds of known constitution are necessary. In this respect the method resembles that for the determination of methyl groups attached to carbon.

Since acetone resembles other methyl ketones in its quantitative formation of iodoform with a hypoiodite, and as these are decomposed only slightly with permanganate it is necessary, with substances of unknown constitution, to determine the acetone in a control test as acetone-*p*-nitrophenyl hydrazone, which is identified by its melting-point (149·5° C.). Thus, the first 5 ml. of distillate are mixed with 7 ml. of a solution of 0·07 gm. of *p*-nitrophenylhydrazine in 7 ml. of 50% acetic acid. The acetone-*p*-nitrophenylhydrazone crystallises out in yellow needles ; sensitivity, 0·004%. If the solution is more dilute, a brownish substance is precipitated, which has a different melting-point.

It is also to be noted that acetone is formed in considerable amounts from compounds which contain no *iso*propylidene groups, *e.g.*, thymol, terpin hydrate, *iso*propyl alcohol. *Iso*propyl groups near hydroxyl groups and double bonds especially, tend to form acetone.

Apparatus

This consists of the ozonising apparatus, two round-bottomed flasks with standard ground-in joints, and a condenser with a similar joint (Fig. 73).

Any usual laboratory apparatus may be used for the decomposition

with ozone. It is connected with the inlet tube of the flask K_1, by the standard joint *s*.

The 100-ml. Jena-type glass flasks K_1 and K_2 are exactly alike, and are provided with standard joints *S*. In the corresponding stoppers are the ozone inlet tubes (which end just above the bottoms

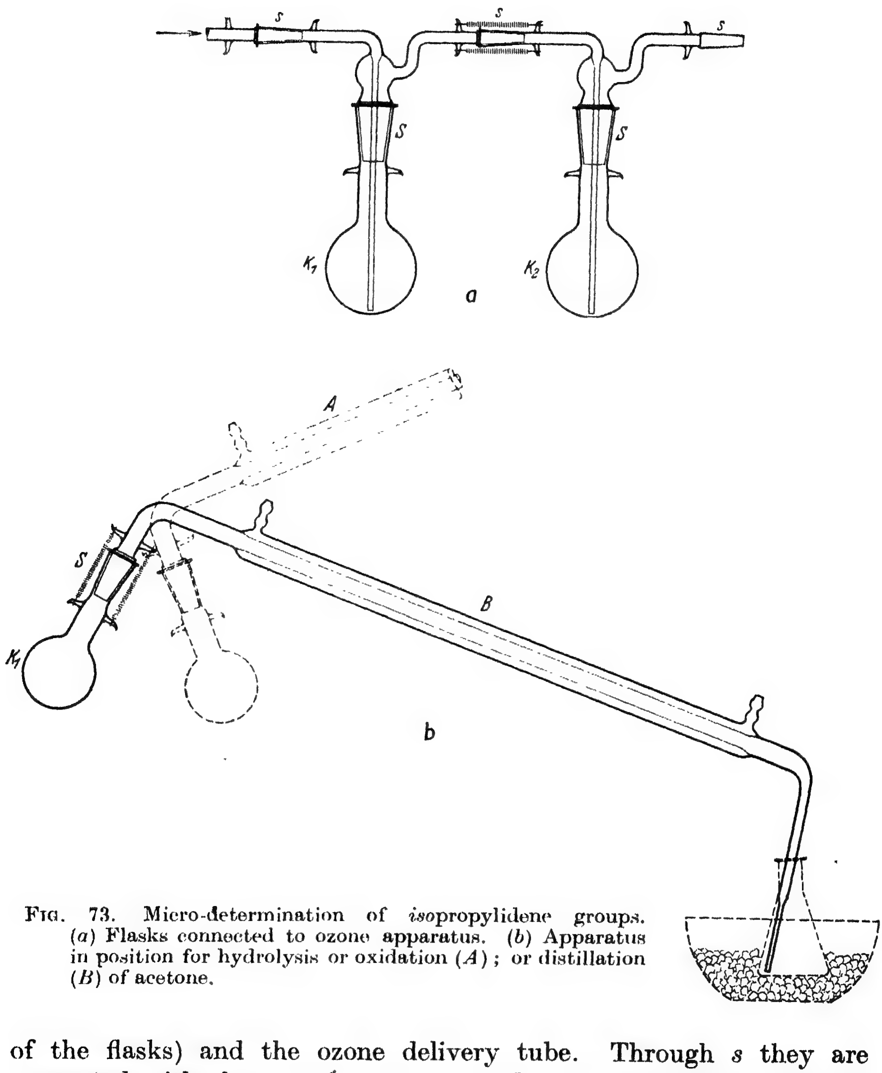


FIG. 73. Micro-determination of *iso*propylidene groups. (*a*) Flasks connected to ozone apparatus. (*b*) Apparatus in position for hydrolysis or oxidation (*A*); or distillation (*B*) of acetone.

of the flasks) and the ozone delivery tube. Through *s* they are connected with the ozone apparatus and with one another. Strong steel springs attached to hooks press the joints (which are moistened with metaphosphoric acid) tightly together, so that no gas leaks.

The Liebig condenser has a 50-cm. jacket and a hollow stopper *S*, which fits into the standard joint of the flask; it is bent through 80° at 6 cm. from *S*. In the position *A* it is used as a reflux condenser; at *B* it is used for distilling off the acetone without breaking the

connexion with the flask. To avoid loss of acetone at a second joint, the condenser is shaped at the outlet end as an adapter.

Reagents

Acetic Acid. 99–100% ; as used for the determination of the Wijs iodine value.

Sodium Hydroxide. 2 *N*.

Sulphuric Acid. 2 *N*.

Sulphuric Acid. 1·0 *N*.

Iodine Solution. 0·05 *N*. Twelve grams of potassium iodide, free from iodate, are dissolved in a little water in a 1-litre graduated flask, 4·6 gm. of iodine are added and the solution is made up to the mark ; the solution is kept in a brown bottle, and the factor is determined after one day.

Sodium Thiosulphate Solution. 0·05 *N*. Since this solution is unstable and is used solely for titrating back unused iodine, only a small stock is prepared. Into a 250-ml. graduated flask are pipetted 125 ml. of exactly 0·1 *N* thiosulphate solution ; this is made up to the mark with boiled water, free from carbon dioxide. In order to increase its stability 0·1 gm. of sodium carbonate is added and the solution is kept in a well-closed stock bottle. The solution can be standardised against 0·05 *N* potassium dichromate solution after one day.

Potassium Dichromate Solution. 0·05 *N*. Exactly 250 ml. of standardised 0·1 *N* potassium dichromate solution in a 500-ml. graduated flask are diluted to the mark with water. After 12 hrs. the solution is checked by titration in duplicate against accurate 0·1 *N* sodium thiosulphate solution. The solution keeps unchanged for years.

Potassium Iodide Solution. 5%.

Starch Solution. 1%. A paste containing 1 gm. of soluble starch in 10 ml. of cold water is poured into 90 ml. of boiling water.

Potassium Permanganate Solution. 1·0 *N* ; 16 gm. in 500 ml. of water.

Metaphosphoric Acid is prepared from phosphorus pentoxide and a few drops of water.

Water used for reagents, rinsing or washing is distilled twice.

Procedure

Determination of the Factors of the Solutions. From a clean, 10-ml. microburette containing the standardised 0·05 *N* potassium dichromate solution 7 ml. are drawn off into a 100-ml. conical flask having a ground-in stopper, 20 ml. of water and 2 ml. of the colourless 5% potassium iodide solution are pipetted into it, and the mixture is acidified with 5 ml. of 2·0 *N* sulphuric acid and allowed to stand for 2 mins. in the closed flask. The 0·05 *N* sodium thiosulphate to be standardised is titrated into it from a second microburette rapidly, until a pale brown colour results ; 3 drops of starch solution are added, and the solution is further titrated until it is just decolorised. From

the volume (read to $\pm$ 0·01 ml.) the factor for this thiosulphate solution is calculated.

If the factor in at least two titrations is obtained accurately to within $\pm$ 0·2%, the 0·05 N thiosulphate is used to standardise the iodine solution as above, except that iodine solution without potassium iodide is used.

Hydrolysis is used when the *iso*propylidene group is attached to oxygen. Sufficient material to yield 1·5–2·5 mgm. of acetone is weighed (from the long-handled weighing tube, p. 73) into the clean dry flask K_1; 10 ml. of 1·0 N sulphuric acid and a few pieces of pumice are then added, and the condenser is fitted in position. The flask is protected by an asbestos wire gauze on a ring, so that the solution just boils. The acetone is usually split off after a few minutes, but as a precaution the solution is boiled for 10–15 mins.; it is then allowed to cool.

Ozonisation is used when the *iso*propylidene group is attached to carbon. The substance is dissolved in the flask in 3 ml. of 99–100% acetic acid. It is important that it should dissolve completely, and the solution may be warmed. Substances insoluble in acetic acid are ground very finely in an agate mortar with the acid, and the resulting dispersion in the acid is ozonised. Oils and liquids are weighed as described on p. 18, or in a small platinum boat. Liquids with high vapour-pressures are weighed by Pirsch's method (p. 202), and the capillary is afterwards crushed with a glass rod in the acetic acid.

The ozone apparatus is set in action with an oxygen current of 20 ml. per min.; the Siemens-Halske ozoniser produces under these conditions oxygen containing 3·2% of ozone. The standard joint S of the flask K_1 is moistened with metaphosphoric acid and secured with a steel spring, and in the second flask K_2 are placed 3 ml. of water; the inlet tube (Fig. 73, a) is connected with the delivery tube of the first flask, and the connexions are secured with steel springs. The flask is then connected at s with the ozoniser, and the second flask K_2 is cooled with melting ice.

After 2–3 hrs. the apparatus is disconnected, and the inlet tubes of both flasks are rinsed with about 10 ml. of water and removed. The contents of K_2 are then rinsed with about 10 ml. of water into the acetic acid solution in K_1, the acid is neutralised with 16 c.c. of 2 N sodium hydroxide, and 5 ml. of N potassium permanganate solution, and a few pieces of pumice, are added. The condenser (the joint of which has been moistened with metaphosphoric acid) is clamped in position A, the solution is boiled for 10 mins. to decompose the ozonide and to oxidise decomposition-products which might affect the titration; care must be taken that the water is running freely through the condenser. After this some permanganate should still be present; if not, 5 ml. more must be added after cooling, and the boiling repeated.

Distillation. The condenser and flask are then clamped in position B, without disconnecting, and a stoppered 100-ml. conical flask, in

which 10 ml. of water have already been cooled in melting ice, is placed under the condenser (Fig. 73). The adapter is not at first immersed in the water, because then the water present will rise into the condenser during the cooling of the distillation flask ; only when the acetone begins to distil over is the adapter dipped into the water. When 20 ml. of distillate have been collected, the receiver is lowered, the adapter rinsed with 2–3 ml. of water, and the conical flask stoppered.

Titration. The acetic acid content of the distillate corresponds with a maximum of 30 ml. of 0·1 *N* sodium hydroxide ; it is immediately made alkaline with 5 ml. of 2 *N* sodium hydroxide and mixed with 10 ml. of 0·05 *N* iodine which is added rapidly, drop by drop, from a micro-burette. In the cold, the formation of iodoform is slow, and the closed flask is allowed to stand for 15 mins. with frequent shaking. To complete the determination acidify with 10 ml. of 2 *N* sulphuric acid, and titrate the unused iodine after 2 mins. with 0·05 *N* sodium thiosulphate solution, using starch as indicator.

New reagents and flasks and those which have not been used for some time, are checked by an exactly similar titration, using twice-distilled water.

Calculation

Because 1 molecule of acetone uses up 6 atoms of iodine, 1 ml. of 0·05 *N* iodine solution corresponds with 0·484 mgm. of acetone or 0·3505 mgm. C_3H_6. Log 0·3505 = $\bar{1}$·54469.

log (% C_3H_6)=log (ml. 0·05 *N* I_2)+log (factor)+2−log (mgm. sample).

Examples :

(*a*) *Iso*propylidene attached to oxygen :

Diacetone-mannose, $C_{12}H_{20}O_6$; mol. wt., 260·15.

Theory, 32·34% C_3H_6.

mgm. substance.	ml. 0·05 *N* Iodine.	% C_3H_6 found.
8·148	7·47	32·12
7·752	7·01	31·69

(*b*) *Iso*propylidene attached to carbon :

Dimethyl acrylic acid, $C_5H_8O_2$; mol. wt., 100·06.

Theory, 42·00% C_3H_6.

mgm. substance.	ml. 0·05 *N* Iodine.	% C_3H_6 found.
7·439	8·30	39·10

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DETERMINATION OF THE NUMBER OF DOUBLE BONDS BY CATALYTIC MICRO-HYDROGENATION

History and Principle of the Method

Two methods have been suggested, viz. :

1. The volumetric measurement of the consumption of hydrogen at constant pressure in a micro gas-burette.

2. The manometric measurement of the decrease in pressure at constant volume, due to the consumption of hydrogen. Ordinary manometers measure the decrease in pressure in relationship to the atmosphere and, because of pressure fluctuations (especially with measurements lasting over long periods), they are at the best only approximate. The Warburg differential manometer (as used for metabolism experiments), on the other hand, measures the decrease in pressure as compared with a second vessel of approximately the same size, and is much more accurate.

The first volumetric method was due to Smith,[1] who worked with about 2 mgm. each of material and of platinic oxide, and a 2-ml. burette. If corrections are made for different solvents (as determined by control experiments) the maximum error is about $\pm 1\%$. This method, as improved later by Slotta and Blanke,[2] has an error of 0·5%.

Manometric methods have frequently been described (see Hyde and Sharp,[3] and Kautsky and Baumeister [4]). Warburg [5] and Willstaedt [6] also carried out many hydrogenations in simple manometers. The amounts of hydrogen so determined were usually between 0·2 and 2·0 ml., and the methods are undoubtedly quite suitable for the determination of only a few readily hydrogenated double bonds ; their simplicity is a great advantage in research on orientation, for routine investigations, and to ascertain the best conditions for the hydrogenation of complicated compounds. Moreover, the simple manometers are relatively very cheap, easily obtainable, and easily adapted for the purpose of hydrogenation.

For the accurate determination of the number of double bonds of highly unsaturated or very slowly hydrogenated substances (*e.g.*, having over 10 double bonds and requiring longer than 3–5 hrs.), the accuracy of the simple manometric method is too low. Kuhn and Möller [7] therefore used a differential manometer, which measures the hydrogen used up by the substance against that used by a comparison substance under exactly similar conditions. The accuracy so attained is such that even after hydrogenation for 40 hrs. the error is only $\pm$ 0·5%. An improved version of this method is described below. The apparatus may also be used for the direct manometric micro-hydrogenation of substances which contain only a few double bonds. In the comparison vessel are then placed exactly the same amounts of solvent and catalyst as in the hydrogenation vessel, but without the comparison substance ; the error, however, is somewhat more, viz., $\pm$ 1–2%.

Apparatus (Figs. 74, *a* and *b*).

The apparatus is distinguished from the usual Warburg differential manometer by the three-way cock *Ha* in which an outlet cock *Hb* is inserted ; by the removable filling tube *R* for the manometer liquid ; and by the construction of the vessel *G*.

The vessels *G* are of Duroglass ; each has a long, wide ground-in joint and two limbs (diameter, 6 mm.), each making an angle of 30° with the central axis. One (*Ga*) is sealed, and the other opens into the horizontal trough *Gb* (dimensions, 20 × 10 × 70 mm.). The joint is such as to allow free overflow of liquid from the appendage *Ga* into the trough, and *vice versa*, on tilting the whole apparatus through about 45°. The joint fits into the hood *S*, to which is attached the displacement vessel *Sa*, which serves to reduce the large volume of *S*. The joint should be outside the vessel, to avoid contact between the solvent and the grease on the stopper during the transfer operation.

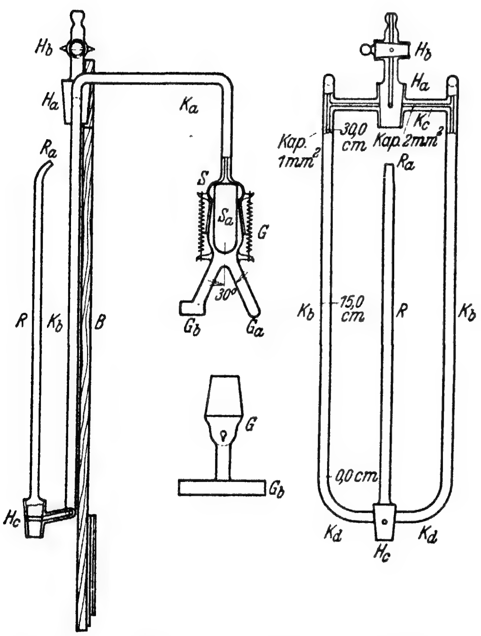

FIG. 74*a*. Micro-hydrogenation apparatus ; side- and front-views.

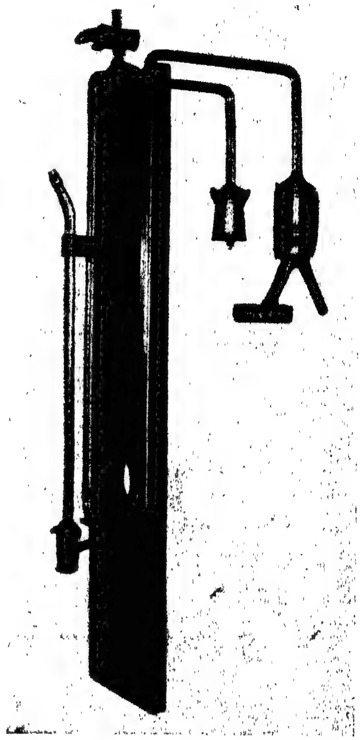

FIG. 74*b*. Photograph of micro-hydrogenation apparatus.

The hoods *S* are connected with the measuring capillaries (*Kb*) of the manometer through the connecting capillaries *Ka*, which are bent twice at right angles. The manometer is fastened to a wooden board, which is inserted in the eccentric rod of the shaking device by means of a bayonet-joint. The measuring capillaries *Kb* are graduated from zero to 30·0 cm., in millimetres, and are connected with one another

so as to form the manometer through a U-shaped capillary, *Kd*. In the middle of this a short capillary branches downwards to the joint *Hc* of the removable filling-tube *R*. The capillary portion slopes slightly downwards, and is as short as possible. The measuring capillaries are also connected by the capillary *Kc*, just above the mark 30, by means of the three-way cock (*Ha*). The capillary *Kc* has a cross-section of 2 sq. mm., whilst that of the others is only 1 sq. mm. The third arm of the cock *Ha* leads to the outlet cock *Hb*, which communicates with the atmosphere. Both *Ha* and *Hb* have bores of 2 sq. mm. cross-section.

Apart from the difficulty of measuring accurately the volume of hydrogen, problems have arisen from the necessity for thoroughly

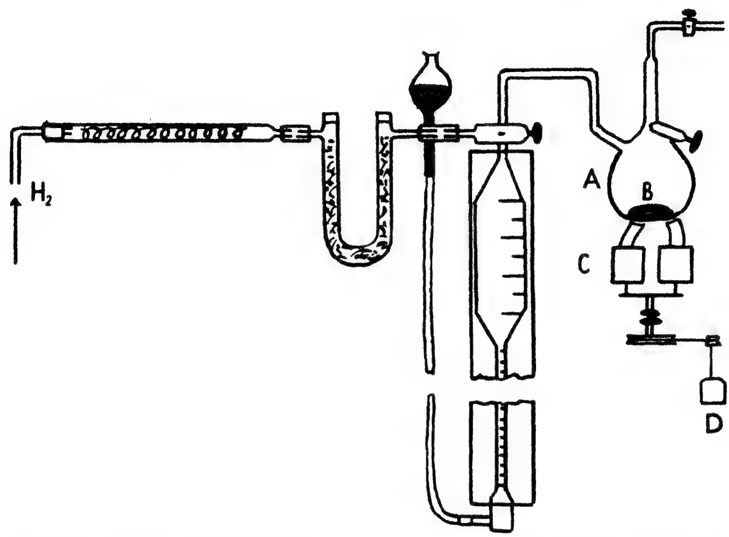

FIG. 75. Micro-hydrogenation apparatus, showing improved method of stirring.

shaking the fragile and complicated apparatus. Prater and Haagen-Smit [8] in particular, have designed a complex apparatus which is mounted rigidly on a support which is oscillated by an electric motor. Johns and Seiferle [9] use a similar device for their simpler non-compensating type of apparatus, but the error involved is $\pm$ 2%. Possibly the best method is that of Weygand and Werner,[10] who agitate the contents of the flask (*A*, Fig. 75) by means of a glass-covered iron stirrer (*B*) which is rotated by a revolving electromagnet *C* operated by a small electric motor *D*. This enables the remainder of the apparatus to be arranged more conveniently and in a stable position.

Calibration. The connecting capillary *Ka* is cut just above the hood *S*, and two calibrations are made as follows : (1) The vessel *G*, with hood *S*, and the attached part of *Ka* (using water). The vessel *G* with the hood *S* is weighed empty, then *S* is taken off, *G* is filled to the top with water, and the hood replaced very quickly (but carefully) so

that the vessel, hood and attached capillary are quite full of water. If they are not, the vessel G is warmed, with tapping, in a boiling water-bath and, after all the air has been driven out and water emerges from the capillary, water is sucked back through a bent capillary during cooling. This operation is to be avoided if possible because, during cooling, the ground-in parts are easily pressed too tightly together and other errors are involved. A little water is now removed from the capillary, the position of the meniscus is marked, and the whole is weighed. The capillaries are then re-fused. (2) The connecting capillaries Ka and measuring capillaries Kb, up to the 15·0-cm. line and including the horizontal capillary piece Kc leading through the three-way cock Ha (using mercury). The apparatus is inverted, the three-way cock Ha is closed (but not greased), and mercury is poured through the hood S till the 15·0-mark of the water-meniscus is reached. The capillary piece Kc is filled through Ha, the level of the mercury in Kb is read, and the mercury used is weighed. The deficit up to the 15·0-cm. line is finally calculated from the cross-section of the capillary, which is determined by the mercury method. The error in the calibration of the vessel is $\pm$ 0·1% ; that for the capillary cross-section is somewhat higher.

Reagents

Hydrogen, prepared electrolytically, is suitable. Traces of hydrogen sulphide and oxygen are removed in a large spiral wash-bottle containing alkaline plumbite solution ; 3–4 gm. of lead chloride are dissolved in 100 ml. of 20% sodium hydroxide solution. A tube containing granulated calcium chloride serves for drying, and cotton-wool in a tube holds back calcium chloride dust; the use of hot palladium asbestos is unnecessary. A set of capillaries regulates the current of hydrogen ; and the pressure is measured by a mercury manometer. The apparatus for purifying and drying has fused joints so far as is possible ; rubber connexions are avoided.

As **solvents,** *acetic acid* (A.R) and *alcohol* (A.R.) may be used without further purification. *Hexahydrotoluene* (A.R.) should be shaken repeatedly with sulphuric acid (A.R.) until it gives no reaction in a platinic oxide and hydrogen micro-test. The acid is renewed twice daily at first, then daily, then every two days, and finally weekly. *Dekalin* is shaken repeatedly for short periods with 5% oleum ; the acid is, however, somewhat coloured, even when the dekalin is already saturated as shown by the platinic oxide and hydrogen test. The hydrocarbons are finally carefully fractionated under reduced pressure. *Cyclohexane* (purified as for hexahydrotoluene), *chloroform, tetrachloroethylene, cyclohexanol* and *octyl alcohol* have also been used successfully as solvents after suitable purification.

Reduced platinic oxide and **palladium oxide** saturated with hydrogen are prepared according to the directions of Adams and Shriner,[11] and the support-catalyst (platinum on silica gel) by the method of Köppen [12]

(see also Slotta and Blanke [2]). It can only be mentioned here that it is very difficult to state in advance which conditions are suitable for a class of compounds or for a single compound, or even which are most favourable. With unknown substances, therefore, several preliminary hydrogenations under various conditions may have to be made.

Procedure

The purity of the substance to be hydrogenated is of the greatest importance. It is frequently observed that substances which give a perfect combustion can only be hydrogenated satisfactorily after a further purification, which does not alter the values of the combustion values.

Sorbic acid is almost exclusively used as comparison substance, in preference even to Willstaedt's azobenzene.[6] The commercial product is purified by alternate crystallisation from dilute alcohol and sublimation in a high vacuum. The preparation is stored in a high vacuum over tablets of potassium hydroxide. The following are the criteria of an ideal comparison substance : (1) Even with a very weak catalyst it must be hydrogenated with a uniform and average velocity. (2) It should show no increased absorption of hydrogen even with a very strong catalyst.

The weight of substance taken must be adjusted according to the consumption of hydrogen by the comparison substance, so that too large a pressure difference does not result at the end of the hydrogenation. The initial velocity of hydrogenation of material and of the comparison substance should be of the same order ; then the amount of substance may be so chosen that its consumption of hydrogen corresponds with a pressure difference of up to 50 cm. If the initial speeds differ too greatly, the amounts of substance taken must be correspondingly varied. It may be preferable in such cases to omit the comparison substance, especially if only very small amounts are available ; the subsequent manipulation is not thereby altered.

The substance to be analysed (0·5–20 mgm.) and 2–3 mgm. of sorbic acid are weighed in long-handled weighing-tubes and placed in the portions *G* of the apparatus, and the catalyst is weighed into the trough. Both vessels should contain the same amount to within $\pm$ 2%. The solvent (1·50–3·00 ml.) is then pipetted into the trough, and the joint is greased, inserted into the hood, and held with spiral springs. The freshly greased filling tube *R* is connected with the capillary *Kd* through the joint *Hc* ; care must be taken that the narrow bore of the stopper is quite free from grease. The mouth *Ra* is connected up by pressure-tubing treated with paraffin wax *in vacuo,* so that the system may be alternately evacuated to about 20 mm. Hg and filled with the purified hydrogen. The cock *Ha* should previously have been turned so that both vessels are in communication, the outlet cock *Hb* being closed. During evacuation the apparatus is well shaken by hand to remove all dissolved air. The evacuation and admission of the

hydrogen are repeated about six times. After the last filling with hydrogen there should remain a small excess pressure of about 50 mm. Hg. Finally, the filling tube R is closed to Kd by turning through 180°, and the tubing is removed from its mouth. With readily volatile solvents, the vessels must be suitably cooled to avoid an alteration in volume through loss of solvent.

The apparatus is now placed in the thermostat at 25° ± 0·02° C. and shaken well for about 1 hr. With support-catalysts, the time required for saturation with hydrogen is usually shorter; with platinum and palladium oxide catalysts it may be several hours. The shaking must be so vigorous that both solvent and catalyst are thrown forcibly against the upper wall of the trough. On quickly turning the outlet cock Hb, the excess hydrogen pressure is relieved. The filling-tube is now filled with an accurately measured amount of absolute alcohol (as manometer liquid) which is admitted carefully through Hc into the capillaries. With care, no bubbles of gas need enter the capillaries. The outlet cock Hb is then quickly opened and closed once more, so that the measuring capillaries are filled just above the 15-line. The exact adjustment is finally made by removal of liquid by inserting a wad of cotton-wool into the filling tube. No appreciable change of pressure as compared with that of the atmosphere should be evident. After this has been checked, R is closed against Kd by turning through 180°, and is sealed with mercury. This is absolutely necessary, because the alcohol slowly dissolves the grease. The three-way cock is finally turned through 90°, so that the vessels are no longer in communication and the apparatus can be used as a differential manometer. The final adjustment to constant pressure is then made.

The apparatus is taken out of the thermostat and tilted repeatedly, so that the sample and comparison substance are brought into contact with the catalyst in the trough. The first reading is taken when the substance is almost completely hydrogenated; this is usually rapid with sorbic acid. The pressure differences at suitable intervals of time are then read, until constant pressure is reached again. The standard of constancy of pressure is naturally relative, and the frequency of the tests has to be adjusted according to the rate of hydrogenation.

Calculation

At the beginning of the experiment, when the pressures in both vessels are the same, the difference of level $h = 0$; the volumes of gas V_x and V_t, however, are different. For simplicity it is assumed that the volume of hydrogen from the unknown substance is the larger, and that its rate of hydrogenation is much lower; this is usually the case. If the comparison substance has now absorbed the volume a_t of hydrogen (which should completely hydrogenate it), and if the pressures have again been equalised, so that the difference of level $h = 0$ again; then the unknown (x) substance has absorbed the volume (say) a'_x of hydrogen. Then:

$$\frac{a'_x}{V_x} = \frac{a_t}{V_t} \tag{1}$$

If the unknown substance is now completely hydrogenated, the difference of level h is positive in relation to V_x. If the volume of hydrogen which corresponds with this difference is called a_h, then the sum of a^x and a_h is equal to the volume a_x of the hydrogen absorbed by the substance at the completion of hydrogenation ; thus

$$a'_x = a_x - a_h \tag{2}$$

Substituting for (2) in (1) :—

$$\frac{a_x - a_h}{V_x} = \frac{a_t}{V_t}$$

or

$$a_x = \frac{V_x . a_t}{V_t} + a_h \tag{3}$$

The ratio of the volume a absorbed to the molecular volume W of the hydrogen is the same as the ratio of the weight of material E to the molecular weight M of the substance to be hydrogenated ; thus, $a : W = E : M$ for one double bond per molecule. If the substance has n double bonds per molecule, then :—

$$\frac{a}{W} = \frac{E . n}{M}, \text{ or } a = \frac{W . E . n}{M},$$

and

$$a_t = \frac{W . E_t . n_t}{M_t}$$

and

$$a_x = \frac{W . E_x . n_x}{M_x}.$$

Substituting in (3) :—

$$\frac{W . E_x . n_x}{M_x} = \frac{V_x}{V_t} . \frac{W . E_t . n_t}{M_t} + a_h,$$

and therefore :—

$$n_x = \frac{M_x}{E_x}\left[\frac{V_x}{V_t} . \frac{E_t . n_t}{M_t} + \frac{a_h}{W}\right].$$

a_h is calculated from the difference of level h and the dimensions of the particular apparatus. Now since the correction a_h/W should be only 20% of the first term in the brackets, while the total pressure is not finally decreased at the end by more than 5% of the original, one can therefore substitute for a_h the expression for the volume absorbed which was given by Warburg [5] for his differential manometer. V_x is then the volume of the " test vessel," and V_t that of the " compensation vessel." Under the given conditions the error of the formula is $\pm$ 0·3%. Since, also, the absorption coefficients of hydrogen in the solvents used are very low and the volume of solvent is, at a maximum, 6% of that of the gas, the corresponding terms of the Warburg formula need, therefore, not be considered. The expression :—

$$n_x = \frac{M_x}{E_x}\left[\frac{V_x}{V_t} \cdot \frac{E_t \cdot n_t}{M_t} + \frac{h}{W}\left(1 + \frac{A \cdot P}{2V_t}\right) \cdot \left(\frac{V_x}{P} \cdot \frac{273}{T} + \frac{A}{2} \cdot \frac{273}{T}\right)\right],$$

is obtained, in which A is the cross-section of the capillary, T the absolute temperature and P the normal pressure of the manometer liquid used, which may be expressed by the specific gravity s. Thence, the formula may be still further reduced :—

$$n_x = \frac{M_x}{E_x}\left[\frac{V_x}{V_t} \cdot \frac{E_t \cdot n_t}{M_t} + h \cdot \frac{273}{T \cdot W}\left(1 + \frac{A \cdot s}{2 \cdot 1{\cdot}034 \cdot V_t}\right)\left(\frac{1{\cdot}034 \cdot V_x}{s} + \frac{A}{2}\right)\right] \quad . \ (4)$$

For negative values of h, and when a_x is smaller than a_t, the expression for a_h must be so altered that V_x and V_t are interchanged, because $(V_t - h)$ is now positive. V_t thus, is now the volume of the "comparison vessel" and V_x that of the "compensation vessel" in Warburg's terminology. The numerical alteration of the expression is not great, but with large values of h and large differences between V_x and V_t it must then be taken into consideration. The expression with negative values is :—

$$n_x = \frac{M_x}{E_x}\left[\frac{V_x}{V_t} \cdot \frac{E_t \cdot n_t}{M_t} - h \cdot \frac{273}{T \cdot W}\left(1 + \frac{A \cdot s}{2 \cdot 1{\cdot}034 \cdot V_x}\right)\left(\frac{1{\cdot}034 \cdot V_t}{s} + \frac{A}{2}\right)\right] \quad . \ (5)$$

If one hydrogenates without the comparison substance, the first term in the brackets is zero, because $E_t = 0$, and :—

$$n_x = \frac{M_x}{E_x} \cdot h \cdot \frac{273}{T \cdot W}\left(1 + \frac{A \cdot s}{2 \cdot 1{\cdot}034 \cdot V_t}\right)\left(\frac{1{\cdot}034 \cdot V_x}{s} + \frac{A}{2}\right).$$

Example :

Hydrogenation of very pure β-carotene with platinic oxide (10·1 mgm.) as catalyst and dekalin and glacial acetic acid (1 : 1) as solvent, using sorbic acid as comparison substance ; volume of solvent, 2·00 ml.

General conditions of experiments :—

$T = 25{\cdot}00° \pm 0{\cdot}02°$ C.
$W = 22504$ ml.
$s = 0{\cdot}789$ (ethyl alcohol, 18° C.).
$V_x = 33{\cdot}169 - 2{\cdot}00 = 31{\cdot}169$ ml.
$V_t = 31{\cdot}618 - 2{\cdot}00 = 29{\cdot}618$ ml.
$A = 0{\cdot}01016$ ml.

Substituting these values in (4) and (5) :—

$$n_x = \frac{M_x}{E_x}\left[1{\cdot}052 \times \frac{E_t \cdot n_t}{M_t} + h. \quad \begin{cases} + \ 0{\cdot}001445 \\ - \ 0{\cdot}001373 \end{cases}\right],$$

when E is calculated in milligrams and h in centimetres.

Special conditions :—

$$E_x = 2\cdot342 \text{ mgm.} \quad M_x = 536\cdot40$$
$$E_t = 2\cdot338 \text{ mgm.} \quad M_t = 112\cdot06$$

After 3 mins., $h = + 0\cdot15$ cm., $n_x = 10\cdot09$
After 4·5 hrs., $h = + 2\cdot65$ cm., $n_x = 10\cdot94$
After 8 hrs., $h = + 2\cdot65$ cm., $n_x = 10\cdot94$

$$n_x \begin{cases} \text{Calculated} = 11\cdot00 \\ \text{Found} = 10\cdot94 \end{cases}$$

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CHAPTER V

DETERMINATIONS OF PHYSICAL CONSTANTS

MELTING-POINT

It is sometimes necessary to determine the melting-point of a substance on smaller amounts than the 1 mgm. or so used in the ordinary melting-point tube. This is conveniently done under the microscope, with the aid of a heated stage.[1] Such stages are also used to observe sublimation and distillation at ordinary or reduced pressure. They are electrically heated and the temperature is usually read with an ordinary mercury thermometer, which is enclosed in the heater. In the improved apparatus now described, however, the temperature is given in terms of the potential (as determined by a thermo-element) on a millivoltmeter calibrated in ° C. It consists of a heating stage (Fig. 76) a round metal box (diameter, 85 mm.; height, 15 mm.) containing the electric heating coil, and glass windows (diameters 3 and 7 mm.) exactly in the centres of the upper and lower walls. It stands on three insulated feet, and may be attached to any microscope by means of two adjustable clamps.

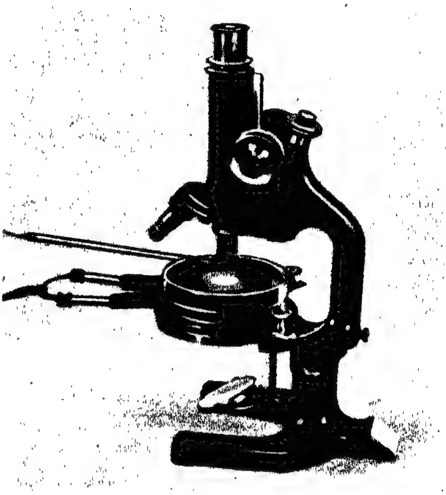


Fig. 76. Micro-determination of melting-point.

Fixed to the heating stage by two pegs is the hotplate, a removable, nickel-plated copper plate, 25 mm. thick. The light passes from the microscope mirror on to the preparation through a central hole of 1·5 mm. diameter. The temperature of the surface of the hotplate is measured with a calibrated copper-constantan thermo-element, and a nickel-plated copper plate (4 × 10 mm.) screwed on to the hotplate at 20–30 mm. from the centre. Both wires of the element (length, 70–80 mm.) are fixed to the hotplate by small screws, the copper wire being in direct contact whilst the constantan wire is insulated from the support by means of small sheets of mica. The surface of the hotplate is protected from air currents by a detachable metal ring, 6 mm. high, which with its four side pieces, reaches to about 5 mm. below the hotplate. It is protected by a removable glass plate having a flat ground top which is placed on the metal ring; the latter has an asbestos covering on its outer wall. The free junction of the thermo-element is

immersed in ice-water. Magnifications of up to about × 135 may be obtained, and usually suffice.

For the determination of the melting-point, the crystals are placed on a slide and are brought exactly over the hole in the hotplate and covered with a cover-glass. Micro-sublimates on cover-glasses (see Kofler [2]) are placed on a slide so that the cover-glass (with the crystals underneath) is above the hole. In order to eliminate air currents glass strips are placed at each side of the cover-glass, and on these is placed a larger cover-glass or slide. Finally, the glass plate is placed on the metal ring.

As soon as the microscope has been focused, the current is turned on through the hotplate and regulated with the help of the rheostat so that the millivoltmeter shows a rise in temperature of 2° C. per min. If the melting-point is known approximately, the temperature should

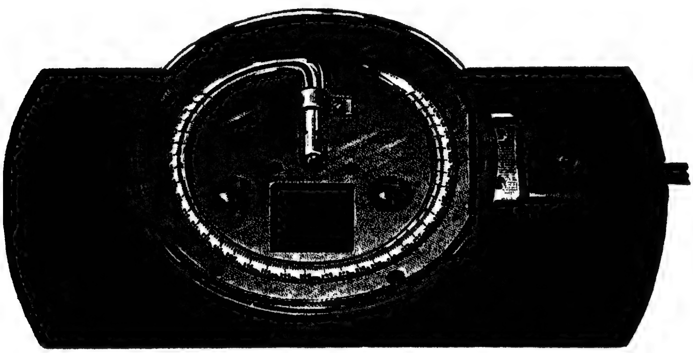


Fig. 77. Electrically-heated microscope stage for micro-determination of melting-point.

be taken quickly up to 10°–15° C. below the melting-point. The error below 200° C. is less than ± 1° C.; at 200°–300° C. it is, at the most, ± 2° C.

Very volatile substances must be fixed before the determination [3] by edging round the cover-glass with cement prepared from casein and milk of lime, or with a commercial glass or porcelain cement. Cracks which appear on setting are closed with a thin, new top layer of cement. The cover-glasses should not be too thin, because they then warp too easily and do not lay flat on the slide, so that high melting-points may result.

Fig. 77 shows a recently evolved electrically heated apparatus in which two thick copper discs sandwich a resistance heating element wound on a mica former. The thermometer is circular (to eliminate the straight stem correction), the temperature is controlled thermostatically, and a black metal plate is provided for the specimen. Alternatively, this plate may be removed, and the sample placed on a cover-glass so that the melting of the crystal may be observed in polarised light through an analyser; this method is particularly useful

with anisotropic compounds (*i.e.*, with most fusible substances), the production of an isotropic liquid at the melting-point being shown by a darkening. By using this principle Zscheile and White [4] were able to obtain an accuracy of $\pm$ 0·04° C. in many cases. Rimington and Symons [5] advise the use of a carbon arc as the source of light, so as to overcome the relatively high absorption of the nicol prisms.

Finally, reference may be made to the method evolved by Morton and Mahoney [6] in which the sensitivity of the ordinary capillary tube macro-method is greatly increased by projecting an enlarged image of the tube and its contents on to a screen. Methods of this kind, of course, only improve the accuracy of observation of the end-point; they do not improve the sharpness of the melting-point itself in the sense that the use of polarised light does, since the latter method indicates precisely the destruction of the crystal lattice structure.[43]

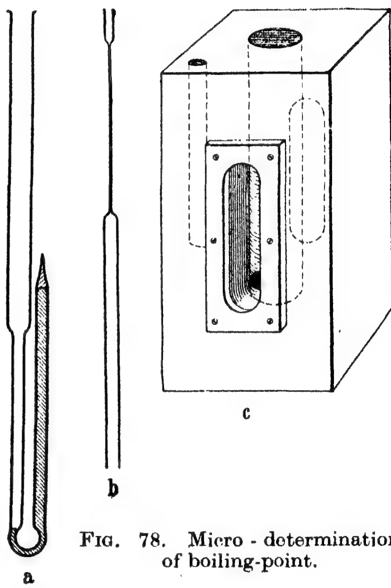


FIG. 78. Micro - determination of boiling-point.

BOILING-POINT

Principles

The simple apparatus shown (Fig. 78) enables the boiling-points of liquid or solids to be determined on 2–5 mgm., over the range 30°–250° C., to $\pm$ 0·1° C. without any correction; this is achieved by means of a special massive aluminium heating-block and a short-range thermometer. The substance is enclosed over mercury and heated to the temperature at which its vapour pressure is equal to that of the atmosphere at the time. The temperature thus determined is the boiling-point.

The method also allows the boiling-point to be determined simply at 760 mm. pressure, even if the current barometric pressure is different. Thus, if the height of the barometer is 750 mm. warming is continued until the vapour pressure has increased by 10 mm., as shown by the difference of the mercury levels. Then the vapour pressure is exactly 760 mm., and the temperature read corresponds with the normal boiling-point. The method is limited to substances which are not decomposed by mercury at their boiling-points; further, it can only be used up to temperatures at which the vapour pressure of mercury is negligible. Up to 250° C. (the boiling-point of benzoic acid) no influence of the vapour pressure of mercury was observed. It is essential that the space above the mercury should be saturated with the vapour of the substance, *i.e.*, that some of the material should still be liquid. The dimensions of the apparatus are such that (*e.g.*,

with water) 1–2 mgm. are sufficient. After successive boiling-point determinations the substance can be recovered.

Niederl and Routh [7] discuss the general errors and corrections involved in these determinations.

Apparatus

An ordinary glass tube (length, 30 mm.; internal diameter, 6–8 mm.) is drawn out in the blowpipe flame to a tube about 25 cm. long and 2·5–3·0 mm. diameter. This narrow tube is drawn out once more to a thin-walled capillary (diameter, 1·0–1·5 mm.); the capillary is finally drawn out over the micro-burner to a hair-fine capillary (Fig. 78, *b*). The tube is now bent to a U-tube (Fig. 78, *a*), so that the wide limb is about twice as long as the capillary limb, by allowing the walls of the tube to fall together in the flame until the bore is 1·5–2·0 mm., and the tube is bent round. If the bore is narrower, adherence of the mercury to the walls is noticeable. The parallel limbs should almost touch one another at the top. The tube is carefully cleaned with warm sulphuric-chromic acids, water, and distilled water, and dried at 110° C.

The heater (*c*) is a massive aluminium block (area, 90 × 65 mm.; height, 170 mm.). Exactly in the centre is a cylinder (diameter, 28 mm.; height, 140 mm.) in which the boiling-point tube is placed during the determination. At the side is a boring for an ordinary thermometer. In the wider front and back surfaces are two large mica windows, through which the level of the mercury can easily be observed. The mica plates, in a brass frame, are screwed fast to the aluminium heating block by means of six screws. When in the course of time they have become cloudy, they can be replaced.

In order to ensure a constant temperature, the whole height of the aluminium block is surrounded with asbestos board, 2–3 mm. thick, which is fastened with wire. On the upper surface similarly, is placed an asbestos board, in which a hole is bored to take the melting-point tube and another for the thermometer. The asbestos cover is essential to ensure the same temperature over the whole of the boring. The block is heated on a tripod with an ordinary burner, and short-range thermometers are used (as in the determination of molecular weight, p. 199) for reading the temperature.

Procedure

One drop of liquid is placed as near as possible to the bend of the boiling-point tube by means of a long glass tube drawn out to a point, and allowed to slide just above the lowest point into the shorter limb. Solids (3–5 mgm.) are dropped from a spatula through the long limb and brought to the same position by tapping. Pure dry mercury is added from a dropping pipette, until it is about 10 mm. below the capillaries in both limbs. Whilst liquids thus collect on the surface of the mercury, solids may occasionally adhere to the walls of the right

limb ; they are easily brought on to the surface of the mercury by carefully warming the limb to the melting-point of the solid and gently tapping it. If, however, only a few particles remain in the limb, the determination is not affected.

The tube is now placed in the heating block (without the asbestos cover) and warmed until the substance begins to boil gently ; any absorbed air then escapes through the capillary. Mercury is now added through the long limb until the upper end of the short limb is filled to the fine capillary with the liquid or liquefiable material, and then the fine capillary is sealed above the junction over a micro-burner. This requires practice, so that a small bubble of air or of a gaseous decomposition product remains behind ; this has no influence on the accuracy of the analysis—on the contrary, it prevents superheating. Finally, by lowering the boiling-point tube to the horizontal position, the mercury is poured out from the long limb up to the beginning of the bend (Fig. 78, *a*). The constriction at the bend prevents the escape of too much mercury and the formation of an air-bubble in the now closed limb.

After working with the long thermometer the corresponding short range thermometer is now moved up to the constriction in the wide limb of the glass tube, about 30 mm. below the substance ; the long limb is pushed through the hole in the asbestos board, and allowed to hang freely in the bore so that the bend is 10 mm. above the bottom of the hollow cylinder and the substance is about 40 mm. below the asbestos board. The air-space between the inner wall of the limb and the thermometer is so small that practically no air current (which would make a correction necessary) is formed. If thermometers of diameter less than 4 mm. are used, glass tubes of 5–6 mm. bore are suitable for the preparation of the boiling-point tube. The heating-rate is adjusted according to the temperature required ; in any case, immediately the first bubble of gas is formed, the flame must be so adjusted that the mercury sinks only slowly in the closed limb. When both mercury columns are at exactly the same height, the temperature is read ; this gives the boiling-point for the current barometric pressure, and it is checked by at least three more readings. For this, the mercury columns are moved now in the one and then in the other direction, by alternately raising and lowering the temperature of the heating block slightly ; the temperature is read immediately the columns stand at the same level. The mean of the readings is taken.

If the normal boiling-point (at 760 mm.) is required, the temperature is raised until the difference in the levels of the mercury in the open and closed limbs is equal to the difference between the current barometric reading and 760 mm. The vapour pressure in the closed limb is then 760 mm. It is sufficient to estimate the difference of level with the help of marks 5 mm. apart on the mica window, or on the boiling-point tube, because a pressure-difference of 1 mm. of mercury

Comparative Boiling-point Determinations

Substance	Boiling-point at 760 mm.*	Boiling-point found †	Barometric height
	Degrees	Degrees	mm.
Ether	35·0	34·5	748
Water	100·0	100·1	760
Toluene . . .	110·0	110·4	760
Pyridine . . .	114·2	114·5	758
Phenol	181·0	180·5	752
	—	180·8	760
Dekalin . . .	188·0	187·8	742
	—	188·2	760
Naphthalene . . .	218·0	217·2	742
Benzoic acid . . .	249·0	249·0	753
	—	249·2	760

* Taken from the *Chemiker-Kalender*.
† Mean of three to five readings.

usually corresponds with a temperature-difference of only about 0·04° C.

In a simpler method, which gives results of sufficient accuracy for most purposes of identification, 1 drop of the liquid is placed in a capillary tube (length, 80 mm.; diameter, 1 mm.), the lower end of which is drawn out to a fine point; this point is sealed so that the liquid encloses a very small air-bubble. The capillary is then attached to a thermometer bulb, which is heated in the same way as in the ordinary melting-point method. At the boiling-point the bubble increases rapidly in volume, pushing the drop of liquid up the tube to the surface of the bath.

The optical projection method outlined on p. 187 [6] has also been applied successfully to the boiling-point determination.

DENSITY

This determination is discussed on pp. 208 and 211, in connexion with determinations of optical rotatory power and molecular refractivity. A useful survey of existing methods is given by Alber.[8] Some of these are of interest, in that they are simpler, though less accurate. Thus, in one a piece of glass tubing of exactly known diameter is fixed on to a glass microscope slide so as to form a cell of approximately 1 ml. capacity. Liquid is placed in it, the height of the level is measured accurately by means of a microscope, and 10 mgm. of sample are added; the increase in height is determined, and thence the volume and density may be calculated with an error of 1%.

In another method small particles of the solid sample are placed in liquids of different densities until one liquid is found in which it remains suspended without either rising or sinking; the densities of the solid and the liquid are then identical. Suitable liquids are prepared from mixtures (in various proportions) of heavy and light non-volatile

solvents (*e.g.*, methyl iodide, bromoform, ethylene bromide, chloroform, nitrobenzene), the densities of which are determined experimentally. It is a good plan to make the final adjustment of balance by warming or cooling the liquid, and calculating the density from the resulting change in temperature.

Conversely, the density of a liquid is determined by adding to it a series of solids of known densities. A much higher degree of accuracy is obtainable by measuring the rate of fall of a solid or of drops of liquid through a column of a liquid in which it is insoluble or immiscible, respectively.[9] The success of this method depends largely on the uniformity of size of the drops, and this is ensured by Rosebury and van Heyningen [10] by the use of a precision pipette working on the plunger principle (see p. 30).

An ingenious but simple micro-pyknometer, suitable for 0·1 ml. or less of liquid, is due to Houghton [42]. It consists of two thin capillaries (external diameter, 0·4 mm. ; internal diameter, 0·1 mm.) joined at an angle of 60° ; a bulb (internal diameter, 0·6 mm. ; length, 2 cm.) is blown in one of them. This V-shaped instrument may be filled with the liquid to be tested by means of a hypodermic needle, and supported on stout pins in a vertical board, on which are graduations corresponding with the arms of the V. It is calibrated by filling it with mercury, weighing it, and noting the level of one meniscus when the other is brought to the zero point of the scale by tipping the apparatus. The change in this level and, correspondingly in the weight, when various quantities of mercury are tipped out, enables the calibration to be completed.

If the pyknometer has a capacity of about 10 cb. mm. ; if it can be weighed to within 10 μ gm. ; and if the meniscus level can be read to within 1 mm. ; then the weighing and the volume errors are less than 0·1%.

MOLECULAR WEIGHTS

Pregl's Ebullioscopic Method

The method does not differ in principle from the macro-boiling-point elevation method ; its development for micro-determinations began in 1912, after the excellent short-range Beckmann thermometer and the corresponding small boiling-vessel with inner condenser became available. With 1·5 ml. of liquid, 3 gm. of small platinum tetrahedra are placed in the boiling vessel, in order to surround the small bulb of the thermometer completely with liquid. It was possible to maintain a constant boiling-point in these experiments only by departing from the principle of heating in stagnant air, and so altering the conditions that warm air in uniform motion passes around the boiling-vessel ; a steady temperature is thus maintained for several minutes.

Apparatus

This (Figs. 79 and 80) consists essentially of a stand on which a rod

adjusted to reduce the flame further. A pressure regulator similar to that employed in the determination of carbon and hydrogen (p. 35) may be also used.

The Internal Condenser is very important for the maintenance of constant ebullition, for if the condensed solvent falls back drop by drop the temperature falls as each drop is returned. This is particularly disturbing with solvents having high boiling-points. If, therefore, the lower point of the internal condenser has much play within the apparatus, its position will be altered each time the latter is touched and therefore, the continuous return flow of the condensed liquid may be broken. Such interference may be avoided by sealing glass beads to the point of the condenser, or better, by sealing a short length of platinum wire to its lowest point. The apparatus must be so protected from air currents that violent movements of a cloth in the immediate neighbourhood cause no change in the temperature registered by the thermometer. For the same reason the apparatus is protected from direct sunlight.

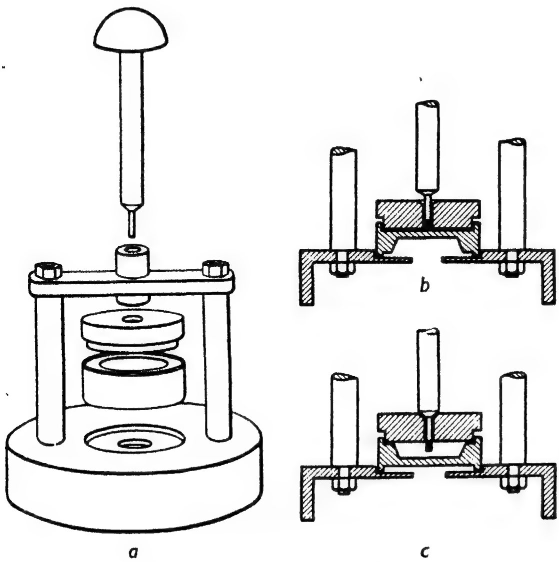


Fig. 81. Tablet press.

An interval of 15 mins. is usually required after the liquid has begun to boil until the mercury thread is steady, as observed through a lens ; the glass of the cylinder requires this time to become uniformly heated. Experience has shown that a constant temperature is more quickly attained, and more accurate values obtained, if boiling is fairly vigorous. Before each reading the tendency of the mercury to stick is overcome by gently tapping with a glass rod covered with a piece of rubber tubing. Once the mercury column is steady to within 0·002° C. for several minutes, weighed tablets of the substance may be introduced.

The Orthofer Tablet Press shown in Fig. 81*a* consists of a thick base plate, with a centre opening in which the two plates illustrated fit, and having two supports for the guide. The upper steel plate, 9 mm. thick, has a central perforation (diameter, 2 mm.), the top of which has a funnel-shaped expansion. The lower part is hollowed out cylindrically by turning, twice on one side and once on the other. As may be seen from Fig, 81, *b*, the central perforation of the top plate is closed by the

planed surface of the bottom plate. The substance is easily inserted in the bore-hole through the funnel expansion, and the piston of the compressor, which exactly fits into the hole, is lowered gently. Finally, a coherent tablet is formed by pressing on the handle of the compressor. In order to remove the tablet the lower plate is inverted (Fig. 81, *c*), and the tablet is pressed into the hollow of the lower plate by a sharp blow on the compressor.

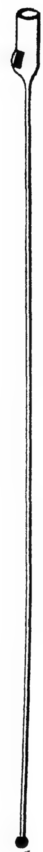


FIG. 82. Charging tube. Two-thirds actual size.

Procedure

To prevent the tablet from sticking in the side-tube it is advisable to place it, after weighing, in a small tube (diameter, 3–4 mm. ; length, 15 mm.) the closed end of which is provided with a small side-pocket in which a glass thread about 150 mm. long and 1 mm. thick is fused (Fig. 82). This is introduced as far as possible into the side-tube, and the tablet released by rotation. The tablets should weigh 7, or, at most, 10 mgm. They are weighed to within 0·01 mgm. only, in weighing tubes such as are used for the determination of nitrogen (p. 73). The first tablet is introduced into the apparatus as soon as the boiling-point has become constant. After dissolution has occurred, the boiling-point rises, gradually at first, then quickly, and finally acquires a maximum value within 2–3 mins. The second tablet is then introduced, so that the actual determination of the molecular weight of the material, introduced in two portions, should take 5–6 mins.

The recovery of the dissolved substance after the determination offers no difficulties ; it is usually possible, after recrystallisation, to obtain 12–15 mgm. of material.

Calculation

$$M = 100 \cdot \frac{K}{L} \cdot \frac{S}{\Delta_t}, \text{ where}$$

S = weight of substance.
K = ebullioscopic constant of the solvent.
L = weight of solvent.
Δ_t = observed elevation of boiling-point.

TABLE FOR THE CALCULATION OF THE MOLECULAR WEIGHT, USING 1·50 ML. OF SOLVENT [36]

Solvent	L (in gms.)	K (in °C.)	$\frac{K}{L}$ (in °C./gm.)	$\log \frac{K}{L}$
Ethyl ether. B.p. 34·6° C., d_{20} = 0·714	1·071	21·6	20·17	1·30466
Acetone. B.p. 56·1° C., d_{20} = 0·792	1·188	17·25	14·52	1·10197
Chloroform. B.p. 61·2° C., d_{20} = 1·488	2·232	38·8	17·39	1·24014
Ethyl alcohol. B.p. 78·3° C., d_{20} = 0·789	1·183	12·0	10·14	1·00602
Benzene. B.p. 80·5° C., d_{20} = 0·879	1·318	25·7	19·50	1·29001
Water. B.p. 100° C., d_{20} = 0·998	1·497	5·2	3·474	0·54078
Glacial acetic acid. B.p. 118·1° C., d_{20} = 1·049	1·573	30·7	19·52	1·29041

Examples :—

Ethyl alcohol as solvent.

$L = 1{\cdot}175$ gm.
$K = 12{\cdot}0°$ C.

Naphthalene, $C_{10}H_8$. *Mol. wt.*, 128·06.

$S = 14{\cdot}44$ mgm.
$\Delta_t = 0{\cdot}112°$ C.
$M = 131{\cdot}7$

Benzene as solvent.

$L = 1{\cdot}34$ gm.
$K = 25{\cdot}7$

Azobenzene, $C_{12}H_6N_2$. *Mol. wt.*, 182·07.

$S_1 = 9{\cdot}67$ mgm. $S_2 = 22{\cdot}57$ gm.
$\Delta_{t1} = 0{\cdot}102°$ C. $\Delta_{t2} = 0{\cdot}245°$ C.
$M_1 = 181{\cdot}8$ $M_2 = 176{\cdot}7$

Glacial acetic acid as solvent.

$L = 1{\cdot}598$ gm.
$K = 30{\cdot}7$

Anthraquinone, $C_{14}H_8O_2$. *Mol. wt.*, 208·06.

$S = 11{\cdot}68$ mgm.
$\Delta_t = 0{\cdot}107°$ C.
$M = 209{\cdot}7$

Rieche's Ebullioscopic Method

Apparatus

Whilst Pregl's method is preferable with solvents of low boiling-point, such as ether, acetone or alcohol, the Rieche apparatus [11, 13] (Fig. 83) is more suitable for solvents of higher boiling-points, such as benzene, water, pyridine or glacial acetic acid, although it entails the use of 4 ml. of solvent and, consequently, of 15–25 mgm. of the substance. Even a transient overheating of the Beckmann thermometer is almost excluded in this case, as the bulb is always surrounded by a mixture of the boiling liquid and of its vapour. The liquid is boiled in the flask *K* and is continually projected, with its vapour, on to the thermometer through the nozzle *D* ; the vapour condenses in the condenser, whilst the liquid falls back into the boiling-flask through the tube *F* ; a small baffle-cone *B*, prevents the liquid from passing in the reverse direction. The liquid is heated over an asbestos-covered wire gauze by a micro-burner, or, if superheating is likely to occur, in a paraffin bath.[12] Superheating is also avoided by the use of platinum tetrahedra weighing 0·3 gm., and the apparatus is protected against air-currents by a cardboard cylinder. As in this apparatus a certain proportion of the substance is always present in the vapour state, the results are usually slightly low, but never by more than 5%.

Procedure

The platinum tetrahedra are placed in the cleaned and dried apparatus, the baffle-cone is introduced, and a thermometer is inserted through a tightly fitting cork and carefully adjusted so that the bulb is completely covered by the inner tubular cylinder *R* ; its lowest point should be about 5 cm. below the upper rim of the apparatus. The whole assembly is then clamped above the asbestos-covered wire gauze and surrounded by a vertical cardboard cylinder. By means of a tared pipette 4 ml. of solvent are added through the side-tube *S*, which is then closed with a cork. The second side-tube is then fitted with a calcium chloride tube, and the dried condenser is so inserted that it does not touch the wall of the tube at any point. The micro-burner is placed exactly under the centre of the boiling-flask 1–3 cm. below the gauze, so that the luminous point of the flame just touches the latter. If the heat is too strong it will be found that the nozzle does not function, and the thermometer reading is not constant. This difficulty is overcome by reducing the size of the flame and raising it nearer to the gauze.

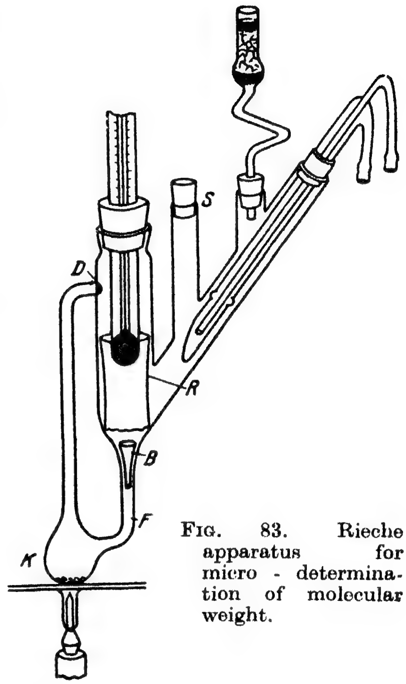


FIG. 83. Rieche apparatus for micro - determination of molecular weight.

When the temperature has remained constant to within 0·002° C. for 5 mins. the first tablet (weight, 15–25 ± 0·1 mgm.) is added through the side-tube *S*. After 2–3 mins. the second temperature reading is taken. When this is constant for a further 2 mins. a second tablet is added, and the third reading of the temperature is taken after this has remained constant for another 2–3 mins. Sometimes (particularly with pyridine or glacial acetic acid) the thermometer bulb is bombarded by liquid which foams and spurts into the descending tube ; this can be avoided by raising the thermometer so that its bulb is 1–2 cm. below the nozzle (*D*).

Notes

In another model [13] the baffle-cone is replaced by a syphon trap, which prevents either hot spray from the boiling-vessel or cold drops from the condenser from coming into contact with the thermometer. About one-third of the boiling-vessel is immersed in a paraffin bath, at 25°–40° C. above the boiling-point of the solvent, and constant boiling results after 25–30 mins. The apparatus is protected from air-

currents by wrappings of cellulose wadding. The nozzle should blow the solvent on to the bulb of the thermometer in blasts which follow one another rapidly.

Other workers [14] have to a great extent overcome the difficulty of obtaining uniform boiling conditions and a constant temperature by the use of an electrically heated platinum coil in the liquid, and a pumping device for avoiding superheating in the neighbourhood of the thermometer bulb. The Beckmann thermometer was replaced by a water-type differential thermometer, which is unaffected by variations in atmospheric pressure, although it can only be used below 100° C.; 5 ml. of solvent and 15–30 mgm. of material are used, and the error is $\pm$ 5%.

Cryoscopic Methods

Principle of the Methods

The observation by Jouniaux [15] that, in addition to its value as a solvent for many substances, camphor produces a high molecular depression of the melting-point, has been used by Rast [16] in the development of a simple but excellent micro-method for the determination of molecular weights. Thus, whilst the melting-point depression of 1 molecule of dissolved substance per kilogram of solvent is only about 1·86° C. in water, it is 40° C. when camphor is used. The method naturally, cannot be used for those substances which decompose below the melting-point of camphor, *e.g.*, many carotinoids, alkannin, etc., but for such substances Pirsch [17] has proposed other hydroaromatic solvents of lower melting-points. These new solvents now make it possible to check the molecular weight obtained by the camphor method. The criteria of a suitable solvent are :—

1. The production of a high molecular depression of the melting-point.
2. Good solvent power.
3. The molecular melting-point depression must be independent of the concentration of the dissolved substance.
4. The substance must give a clear solution and not react with the solvent. The sharper the melting-point, the more accurate is the analysis (*cf.* p. 187).

Incidentally the method also enables the molecular heat of fusion (W) of the solvent to be calculated from the Van't Hoff equation :—

$$W = \frac{\mathrm{R.T^2.M}}{\mathrm{100.K}},$$

where R = gas constant, T = absolute temperature, M = molecular weight of the test substance, and K = molecular lowering of melting-point.

Apparatus

A micro-Kjeldahl flask is filled to the neck with concentrated sulphuric acid, or with water if the melting-point is below 80° C.

Conical melting-point capillaries are drawn out from clean test-tubes in the blow-pipe flame ; they should be about 4 cm. long and the narrow end (which is sealed) should have a bore of 2·5 mm., the bore of the wider end being at least 3 mm. (Fig. 84). Care should be taken, when sealing the narrow end, to produce a nicely rounded hemisphere without forming a point or a heavy drop of glass. For introducing the substance ordinary melting-point capillaries (length, at least 5 cm. ; diameter, 0·5–1·0 mm.) and glass rods 6 cm. long which can easily be passed through the bore of these capillaries, are required. For introducing the solvent, capillaries (length, 5·0 cm. ; diameter, 1·0–1·5 mm.) and glass rods at least 6 cm. long, which are easily passed through them, are required. The capillaries for introducing liquid samples are described on p. 201.

The melting-point is read by a lens, and the thermometer used should cover the range 0°–200° C. in 50° C.-stages. A sensitive thermocouple has also been used for this purpose, and one apparatus of this type is also provided with an electromagnetic stirrer[18] (*cf.* p. 178).

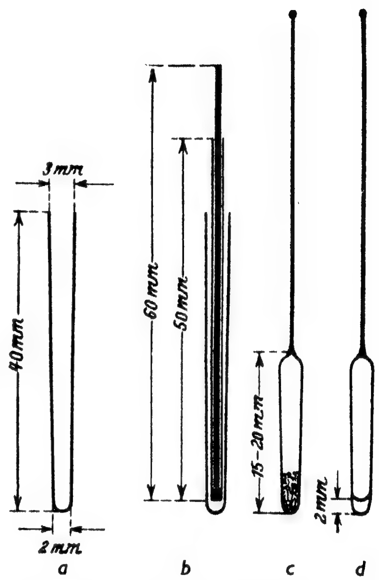


FIG. 84. Micro-determination of molecular weight by Rast's method. (*a*) Original capillary. (*b*) Charging the capillary. (*c*) The sealed capillary before, and (*d*) after fusing the contents.

Difficulties are experienced in this method with dark coloured substances (*e.g.*, ester gums), and in a modified method Aluise[19] submerges a small, sealed glass capsule in the solution of substance to be frozen. If the annular space between the capsule and the side of the containing-tube is only 0·5–1·0 mm. thick, the first appearance of crystals is readily seen with the aid of a lens.

Reagents

Camphor. A.R. ; 10–20 gm. are sprinkled with a little ether, ground to a uniform powder in a mortar, spread in a thin layer on filter paper, and protected from dust (by covering with paper) until the ether has evaporated. The camphor is stored in a wide-necked bottle with a ground-in stopper. A good preparation has m.p. 176°–180° C., and produces a molecular melting-point depression of 38°–40° C.

Solvents suggested by Pirsch[17, 20] are :—

Camphene. M.p., 49° C. Molecular lowering of melting-point, $K = 31°$ C. This has often been found very satisfactory, owing to its good solvent power and high molecular melting-point depression.

Bornylamine. M.p., 164° C. $K = 40{\cdot}6°$ C. In consequence of its

basic character it is particularly suitable as a solvent for alkaloids and basic substances in general.

Camphoquinone. M.p., 199° C. $K = 45{\cdot}7°$ C. This may be used instead of camphor as a solvent for substances of high melting-point.

Camphenilone. M.p., 38° C. $K = 64°$ C.

Dihydro-α-dicyclopentadiene-one-3. Its particularly high molecular depression of freezing-point makes possible the determination of proportionately high molecular weights.

Cyclo-pentadecanone ("Exalton"). M.p., 65·6° C. $K = 21{\cdot}3°$ C. This is a good solvent for azo dyes, many quinones and carotinoids, and especially for sterols and their derivatives. "Exalton" may be used without purification.

Perylene [21] is used for anthraquinones of high melting-point and perylene derivatives ; $K = 25{\cdot}7°$ C.

2, 4, 6-*Trinitrotoluene* [22] is used for polynitro compounds ; $K = 11{\cdot}5°$ C.

Before using a new solvent, its melting-point and the molecular lowering of its melting-point must be known accurately. The former is determined as described on p. 185, taking at least three readings, to within $\pm$ 0·1° C.

To find the molecular lowering of the melting-point, the melting-point of an analytically pure substance of known molecular weight (*e.g.*, naphthalene or benzoic acid) is determined (see p. 202) after adding 10 times its weight of solvent. Several readings are taken to within $\pm$ 0·1° C. The molecular melting-point depression, K, of the solvent is calculated from the equation :—

$$K = \frac{M.L.\Delta_t}{1000.S}, \text{ where}$$

M = molecular weight of the substance added.
L = weight of solvent.
Δ_t = difference between the melting-points of the solvent and solution.
S = weight of substance added.

Procedure

Weighing out Solids (Pregl). The clean melting-point capillary is placed with its opening uppermost in a small, empty counterpoised flask (p. 13), the weight of which is determined to within 0·001 mgm. after a few minutes ; the flask with the melting-point capillary, is counterpoised, so that the rider on the beam reads less than 5 mgm. After weighing, the flask and capillary (which must not be touched again by hand) are placed on a notebook in front of the balance. A small amount (*e.g.*, 0·2–0·3 mgm.) of material is placed on a clean watch-glass and carefully pressed up into the narrow auxiliary capillary, which is then carefully brushed with a marten-hair brush and introduced into the bottom of the melting-point capillary ; its contents are then

expelled by the appropriate glass rod, the auxiliary capillary being raised 2–4 mm. Very hard, granulated materials, which cannot be pressed in, are pushed with a spatula into the slanting auxiliary capillary, and transferred from this to the melting-point capillary by correspondingly tilting the counterpoised flask. After the weight of substance has been determined accurately, the solvent is added in the same way with the help of the wider auxiliary capillary, care being taken not to push this capillary to the bottom of the melting-point capillary because particles of the material already weighed may stick to its edge. It is advisable to add the solvent to the melting-point capillary to a height of 4–6 mm., by pushing it out of the auxiliary capillary, as described, with the glass rod. Sometimes the weight shows that a further charge of solvent is necessary in order to obtain approximately 10 times the proportion of solvent to the substance under investigation. The third weighing, from which the exact weight of solvent is obtained, then follows. The middle of the conical capillary is then sealed by heating over a small flame, and drawn out to a thread about 4 cm. long. The hollow portion, with the weighed charge, should be 15–20 mm. long (Fig. 84).

FIG. 85. Guide tube for viscous liquids.

Oily and Viscous Substances (Pirsch). A glass rod about 0·7 mm. in diameter is sealed exactly in the centre of a small glass tube (length, 80–90 mm.) open at both ends and having a maximum external diameter of 2·5 mm. (Fig. 85). The glass rod projects 1·0–1·5 mm. at one end. By carefully dipping the glass rod sideways into the material to be used, sufficient adheres to it for one weighing. The guide tube is held with the thumb and first finger of the right hand, and the substance is transferred to the bottom of the melting-point tube, which is held vertically. The protecting tube makes it impossible to moisten the side of the melting-point tube. The solvent is then weighed as with solids.

Liquids of High Boiling-point (Soltys). The liquid to be weighed is introduced by means of a glass tube, the end of which is drawn out to a hair capillary, 1·5–2·0 mm. long; this is most simply made by drawing out an ordinary glass tube of suitable dimensions. The hair capillary is filled by dipping it into the liquid concerned, wiped externally, introduced into the bottom of the melting-point capillary in such a manner that the point does not touch the inner wall, and the contents (which should weigh 0·2–0·3 mgm.) are blown out on to the bottom of the capillary (Fig. 86). The second weighing should be carried out quickly, after which the requisite amount of solvent is introduced and the determination continued as already described for

solids ; mixing by rotation in a preliminary bath is inadvisable, as traces of liquid may adhere to the upper end of the sealed capillary.

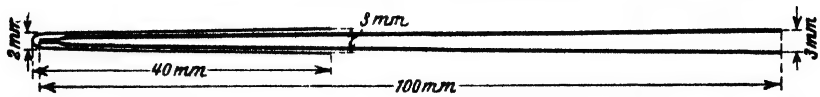


FIG. 86. Method of charging the capillary with liquids of high boiling-point.

Owing to the fact that the solvent absorbs the liquid, mixture is so good that the melting-point becomes constant after once melting and setting in the melting-point apparatus.

Liquids of Low Boiling-point (Pirsch [17]). The solvent is weighed into the melting-point capillary (as on p. 199), and the liquid, weighed in a fine capillary, is added before sealing. The capillary for the liquid (bore, about 1 mm.) is sealed at one end, which is drawn out to a hair capillary 10 mm. long, at a distance of 8–9 mm. from the sealed end. To introduce the liquid, the fine tip of the weighed capillary is immersed in a small dish, in which there are a few millimetres of liquid, so that its wide end projects at least 6 mm. above the surface. The capillary is lifted out of the liquid in warm forceps for a few seconds, and then replaced so that the liquid rises in it on cooling. After sufficient liquid has been sucked up to fill the conical upper part, the capillary is lifted out of the liquid in bone forceps, so that air is sucked back through the hair capillary. The extremely narrow and proportionately high air column thus formed in the hair capillary completely prevents leakage of vapour from the liquid. The capillary is now weighed.

It is then allowed to fall into the melting-point capillary, in which the solvent has been weighed. Conduction of heat to the capillary through the longer melting-point capillary is now prevented by quickly sealing ; if, however, in spite of this liquid enters the capillary, this causes no error, because as experience shows, the vapour cannot diffuse into the melting-point capillary sufficiently quickly. The mixture of the liquids is achieved either by repeatedly immersing the prepared capillary in a bath warmed to the melting-point of the solvent ; or by carefully and repeatedly warming the wide part of the sealed-in capillary and solvent.

Reading the Melting-point. To mix the substance and solvent thoroughly together, a so-called mixing bath is prepared ; water or sulphuric acid (according to the solvent) is warmed to 1°–2° C. above the melting-point of the solvent. The capillary is then held by the glass thread, the whole of the hollow part is immersed in the bath, and the substance is completely dissolved by energetically rotating the mixture. Should observations with a lens show that the substance is not completely dissolved after rotating for a long time, a melting-point determination is useless. If the substance dissolves except for a very small residue, it will probably be dissolved completely using a larger amount of solvent. If it remains undissolved, another solvent must be tried.

After cooling, the glass thread of the capillary is attached, by means of a thin rubber band, to the appropriate thermometer, which is introduced into the melting-point apparatus in the usual way. It is best to heat the apparatus with the pilot flame of a Bunsen burner, so that the temperature rises at a maximum rate of 2° C. per min.; near the melting-point the heating is carried out more slowly, and at a few degrees below this melting-point the contents of the capillary are converted into a turbid liquid in which a crystalline network can be seen with the lens. The network originally occupies the whole of the liquid, but on raising the temperature the crystals vanish from the top, and the last of them, at the bottom, usually disappears with a slight eddying motion. At this point the temperature is read accurately to 0·5° C. The whole is then cooled, and a second check reading is taken.

Calculation

$$M = \frac{1000 \, . \, K \, . \, S}{L \, . \, \Delta_t} \quad \text{(see p. 200).}$$

Example (camphor as solvent):

	Nitrobenzene, $C_6H_5NO_2$	Carbon disulphide, CS_2
Theoretical mol. wt.	123·04	76·1
L	4·599	14·549 mgm.
K	38	31·08° C.
S	0·237	0·807 mgm.
Δ_t	15·7	22·0° C.
Calculated mol. wt.	125	78·3

Osmosis Method

Principles

During an investigation on mushrooms, variations in the vapour pressures of salt solutions were noticed, and it was suggested to G. Barger that these might be the basis of a method of molecular weight determination.

Barger's method [23] is based on the following principle: If solutions of different concentrations are placed in a closed system, solvent passes from the solution which is osmotically weaker to that which is osmotically stronger, by isothermal distillation; ultimately the same osmotic pressure is set up in both. Thus, if a solution of known concentration of the substance to be examined is prepared and tested against comparison solutions of known molecular concentrations, the molecular weight may be determined very accurately. For the determination, small drops of the solution of the substance and of the comparison solutions are placed alternately between small air bubbles

in a tube. The alteration of the distance between the two meniscuses of a drop, owing to isothermal distillation, is determined under the microscope by means of an eyepiece micrometer. The comparison solution which shows no osmotic alteration is used for the calculation of the molecular weight of the substance (see p. 206).

No special apparatus or technique is required, and the method has a wide range of application because of the many solvents and mixtures of solvents which may be used ; these include solvents which are not analytically pure. The only drawback is that rather a long time elapses before osmotic equilibrium is reached. This time depends on the vapour pressure of the solvent ; *e.g.*, with acetone, about 12 hrs. ; with pyridine or water, a few days.

Apparatus

The *measuring capillary* is best prepared from an ordinary glass tube with walls 2 mm. thick and about 15 mm. external diameter. The carefully cleaned tube is drawn out to a diameter of 0·9–1·2 mm. for organic solvents and 1·5–2·0 mm. for water, and cut in pieces about 150 mm. long. The capillaries must be accurately checked before filling ; above all, they must have the same internal diameters.

Any *microscope* having a focal length of about 18 mm., a magnification of × 60–100, and a micrometer inserted in the eyepiece may be used.

Reagents

On account of its excellent solvent power, pyridine is the most suitable solvent ; a mixture of pyridine with acetone, and solvents of low boiling-point (such as ether, acetone, ethyl acetate or alcohol) are also used.

Azobenzene (preferably), cane sugar, benzene and urea are used as comparison substances.

Comparison solutions are prepared [24] after the suitable solvent has been found by a preliminary test. Those prepared from non-volatile solvents are stored in small flasks with ground-in stoppers. Solutions in volatile solvents should be freshly prepared by dilution of a 0·1 *M* comparison solution with the solvent, from a micro-burette (see Table below [24]).

The concentration of the solution of the substance to be examined must be known in order to calculate the molecular weight, and sufficient material to give a 0·1–0·3 per cent. solution is weighed into a graduated flask (capacity, 1–3 ml.). If there is insufficient material for this, it is preferable to determine the percentage by weighing the material and the solvent separately. To calculate the concentration of solution from this, a determination of the specific gravity is necessary ; this is described on p. 208.

Tabulated Azobenzene-Acetone Series of Dilutions [24]

Hypothetical molecular weight	gm. of azobenzene in 10 ml. of acetone	Normality	Dilution	
			ml. acetone	ml. 0·1 M azobenzene solution
100	0·18212	0·1000	—	—
120	0·15175	0·0835	40	8·0
150	0·12140	0·0667	30	15·0
170	0·10712	0·0588	30	21·0
200	0·09105	0·0500	25	25·0
220	0·082773	0·0455	20	24·0
250	0·072840	0·0400	20	30·0
270	0·067445	0·0370	15	25·5
300	0·060700	0·0334	15	30·0
320	0·056906	0·0313	10	22·0
350	0·052029	0·0286	10	25·0
370	0·049217	0·0270	10	27·0
400	0·045525	0·0250	10	30·0

Similarly, the solutions used for polarisation experiments (p. 207) may also be used for determinations of molecular weights.

Procedure

The filling of the capillaries requires some practice ; it is, however, not difficult, and takes only a short time. The measuring capillary is held between the thumb and middle finger, and while one end is covered with the first finger the other is dipped in the comparison solution so that only a small amount of solution enters. The capillary is then raised, turned horizontally, the first finger is removed and, by tilting, the droplet is moved about 3 mm. into the capillary. Similarly, one droplet of the solution of the substance is transferred to the capillary. This is repeated until about 7 droplets, of the two solutions alternately, are in the capillary ; they are allowed to enter it until the last is about 1 cm. from the opening. Finally, with the capillary horizontal, both ends are sealed with a micro-burner. Aqueous solutions are occasionally difficult to slide into the capillary. The end of the capillary farthest away from the solution is therefore gently warmed and then covered with the finger, so that the drop is sucked in on cooling. If very volatile solvents (ether, carbon disulphide) are used, the capillary is best sealed with paraffin wax. The other measuring capillaries are filled with a series of comparison solutions of decreasing concentrations.

The prepared measuring capillaries are mounted at each end on a microscope slide, by means of wax or narrow plastic cellulose strips ; the slides are then labelled (Fig. 87).

For the measurement, the slide with capillary is placed in a Petri dish, and water at room temperature is added until the capillary is just

covered ; it is thus protected from air currents, while the image is made clearer. The microscope is focused on the axis of the capillary, because here both meniscuses of a drop are most sharply defined. The smallest interval between the meniscuses in the axis of the capillary is measured to within 2–3μ by bringing one meniscus exactly to the zero of the micrometer by moving the Petri dish. Thus, in Fig. 87 drops 2 to 6 are measured, and the nearest comparison solution

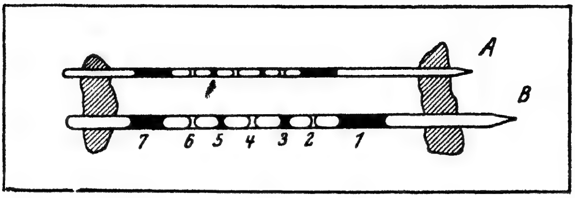


Fig. 87. Osmosis method for the micro-determination of molecular weight.

is then measured. The interval before the next reading depends on the vapour pressure of the solvent used (see above). If the solutions are very dilute the time required is correspondingly longer. The reading is taken for drops 2 to 6 only, because both the outside drops (1 to 7) undergo normal changes owing to evaporation into the adjacent larger volume of air.

The readings which now follow must be taken at the same temperature, so as to ascertain whether the distance between the meniscuses of a drop has become larger or smaller. The series of comparison solutions is chosen so that one just produces an increase and the other a decrease in the distance between the meniscuses concerned ; only in exceptional cases is no change observed. The smallest isothermal distillation thus occurs between the droplets of the solution of the substance, of known weight concentration, and that of the comparison solution, of similar known molecular concentration.

Sometimes, on filling, the droplets pass through the part of the capillary which was moistened previously so that mixture occurs. The observed change is, therefore, somewhat low. However, since we only wish to know which solutions have the same osmotic pressure and do not wish to measure the difference, the sensitivity of the method is only slightly reduced. Rast [25] has modified the method so that only 1 drop each of the solution of the material and of the comparison solution is introduced into the capillary. The changes for both solutions are measured against a line scratched on the slide between 2 droplets. Other modifications of the method also exist.[24, 26-29]

Calculation

$$\text{Mol. wt.} = \frac{\text{Concn. of soln. of sample} \times 10}{\text{Molecular concn. of comparison soln.}}.$$

Example : Molecular weight determinations of grape-sugar

Cane Sugar	Time in Hours	II	III	IV	V	VI	Sum
0·05 *M*.	18	+230	−97	+71	−79	+71	+548
0·10 *M*.	18	+ 26	−18	+25	−31	+30	+130
0·12 *M*.	21	+ 6	− 4	+ 9	− 4	+ 4	+ 27
0·13 *M*.	22	+ 8	+ 3	+ 5	− 1	+ 5	+ 22
0·14 *M*.	22	− 1	0	− 2	+ 2	− 2	− 7
0·15 *M*.	18	− 3	+ 8	0	+ 9	− 4	− 24
0·20 *M*.	18	− 41	+55	−57	+53	−45	−251
0·25 *M*.	18	− 75	+85	−81	+65	−78	−384

(dextrose) against an aqueous solution of cane sugar (molecular weight, 342). Concentration of the grape-sugar solution = 2·502%.

Only the alteration of the meniscus relative to the first reading, after the time in column 2, is given; and not the actual variations in dimensions of the droplets. Of the 5 droplets measured, II, IV, VI are dextrose solutions, and III and V are cane-sugar solutions. The last column gives the sum of the changes for the 5 droplets.

It is seen that the osmotic pressure of the 2·502% dextrose solution corresponds with that of a 0·13–0·14 *M* solution. From this, the molecular weights $= \frac{2{\cdot}502 \times 10}{0{\cdot}13} = 192$; and $\frac{2{\cdot}502 \times 10}{0{\cdot}14} = 179$.

Molecular weight calculated from formula, $C_6H_{12}O_6$, = 180·09.

Bourdillon [29] has devised a method for determining the osmotic pressure of a 0·2-ml. sample, which is particularly useful for substances of high molecular weights. The principle, however, is based on the usual membrane method of measuring osmotic pressures; no correction for specific gravity is involved. The error is about 5% for 0·025 *M* solutions.

OPTICAL ROTATORY POWER

The first attempts to make this determination on the micro-scale were due to Donau,[30] who used capillaries 0·5 mm. in diameter. Fischer [31] and Beutler [32] developed the method as follows :—

Apparatus

Charging *pyknometers* (Pregl type) of about 0·12 and 0·22 ml. capacities (Fig. 88, *a*).

Small 1·5-ml. *weighing-bottles* with ground-in stoppers (Fig. 88, *b*).

Fischer *polarimeter tubes* :—

Diameter	Length	Capacity
1·6 mm.	5 cm.	0·1 ml.
1·6 mm.	10 cm.	0·2 ml.
2·5 mm.	10 cm.	0·5 ml.

Naumann [33] prepares these tubes from black glass with frosted walls, to avoid internal reflections which interfere with the readings.

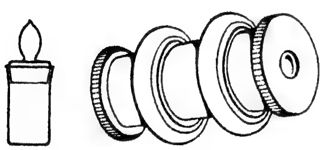
A suitable *polarimeter* is a half-shadow apparatus, with a bi- or tri-partite field of view. Monochromatic light is obtained with the aid of a monochromator which analyses spectroscopically the light from a Nernst lamp ; or preferably from an electric incandescent lamp. This enables measurements to be made with rays of different wave-lengths (*e.g.*, for determinations of rotatory dispersion). For extremely accurate measurements with yellow light (D-line, 589 mμ), a sodium vapour-filled lamp may suitably be substituted for the more expensive monochromator ; for red light, the Siemens cadmium lamp, with or without filter, which radiates principally the line 643 mμ, is suitable.

Another set of gas-filled lamps is now available ; these allow measurements with monochromatic light to be made in almost every part of the spectrum, and are extremely convenient to handle ; it should therefore be necessary to use the monochromator in conjunction with a corresponding source of light in exceptional cases only, *e.g.*, when extreme range of the spectrum or particularly strong light is necessary.

Procedure

Solutions are prepared as follows :—

(*a*) A weighed amount of substance is dissolved in a calibrated measuring flask and the concentration *c* obtained in gm./100 ml. This is preferable if there is sufficient material. If, however, only a few milligrams are available—

a *b* *c*

FIG. 88. Micro-determination of [α]. (*a*) Pyknometer. Actual size. (*b*) Weighing bottle. (*c*) Polarimeter tube.

(*b*) the clean weighing bottle (Fig. 88, *b*) is placed on the balance and weighed after 10 mins. It is then transferred (in forceps or chamois leather) on to a notebook in front of the balance, 3–10 mgm. are carefully placed in it with a spatula, the stopper is loosely inserted, and the bottle is weighed again after 5 mins.

The solvent is allowed to flow carefully from a fine pipette down the wall of the weighing bottle on to the substance ; 0·15 or 0·25 ml. is added, according to the tubes and pyknometers selected. Then the ground-in stopper is fitted in tightly and after 5 mins., the solvent is weighed. If the substance has not dissolved completely the mixture is carefully shaken.

From the two weights the concentration of the solution (gm./100 gm. solution) is found ; to ascertain the concentration *c*, a determination of the density (d^t_4) is necessary.

Determination of the Density (see also p. 190). The charging pyknometer (Fig. 88, *a*) is washed successively with water, alcohol and

ether, dried by means of the pump, cleaned with a chamois leather, and placed beside the balance. Since all weighings must be carried out at exactly the same temperature, a few millilitres of distilled water, and the solution, are placed close together in the balance case. After 10 mins. the empty pyknometer is placed on the balance by means of a fork, and weighed to within 0·01 mgm. 5 mins. later. The pyknometer should not be touched again by hand after this, but is removed with the fork and held in chamois leather while pressure tubing with a mouthpiece is fitted on it. The top is then immersed in the water at room temperature, and this is carefully sucked through to not more than 1–2 mm. above the mark ; the pyknometer is immediately turned horizontally, the tubing removed without compressing it, and the water removed from the outer wall of the tip with filter paper without touching the outlet capillary. The pyknometer is then tilted with the tip downwards through 30°–40°, and water is absorbed from the tip with damp filter paper, until the level is exactly at the mark.

If on filling the pyknometer the water is drawn to more than 1 mm. above the mark, then to enable the water adhering to the wall of the capillary to drain off, the pyknometer is kept tilted for 2 mins., after which water is again sucked up to the mark. With practice no difficulty is experienced in doing this. If the water is drawn to much more than 1 mm. above the mark, the accuracy is affected to such an extent that the drying and filling of the pyknometer must be repeated.

In spite of the rather lengthy manipulation, during which a slight absorption of heat is inevitable, the weighing is carried out after 5 mins. Finally, the solution is weighed with the same pyknometer, and at exactly the same temperature. The error is 0·5 part per 100,000 with a 1-ml. pipette of 1 mm. bore.

Filling the Tube. After the tube has been cleaned with water, alcohol and ether, and dried by the pump, a small glass plate is placed over the end and the stopper is screwed on. The pyknometer is then removed from the balance and held by its upper end, so that the tip is in contact with the bottom of the small tube ; as the solution flows in the pyknometer is raised progressively to avoid the inclusion of air-bubbles. When the solution appears above the edge of the tube, the pyknometer is removed, the second small glass plate is put on, and the cover is immediately screwed on, but not too tightly, because double refraction may then develop in the glass and so impair the uniformity of the field of view and lead to a zero error. As a precaution against this all measurements are repeated after reversing the tube.

After ascertaining that air bubbles are absent, the tube is placed in the polarisation apparatus. Streaks which blur the field of view are due to differences of temperature, and disappear after some time as the temperature equalises itself. By moving the observation tube about in the groove of the polarimeter, the best position for a sharp reading is found. The mean of 6–8 readings, from dark to clear and *vice versa*, is taken. With practice, the error is $\pm$ 0·1° C.

The zero of the apparatus at the time and at the temperature of the measurement is determined by filling the tube with the solvent used.

Calculation

The specific rotatory power $[\alpha]$ is the angle of rotation, α, of the plane of polarised light produced by a solution of 1 gm. of material in 1 ml. when the beam of light passes through a layer 1 dm. long.

If the concentration c (gm./100 ml.) has been determined with the measuring flask, then

$$[\alpha]_D^t = \frac{\alpha . 100}{l . c} \tag{1}$$

where t = temperature of observation, D = wave-length of the light, α = angle in degrees, and l = length of the tube in decimetres.

If the pyknometer has been used, d^t_4, the density of the solution referred to water at 4° C., must first be ascertained. For this, W, the weight in grams of water in the pyknometer at t° C., is divided by the density of the water at this temperature,[34, 36] giving W_4, the volume of the pyknometer in millilitres. The reduced density is :—

$$d^t_4 = \frac{L^t}{W_4} \tag{2}$$

where L^t is the weight in grams of the solution in the pyknometer at the temperature t. For the specific rotation, then :—

$$[\alpha]_D^t = \frac{\alpha . L}{l . S . d^t_4}. \tag{3}$$

α = angle of rotation read.
L = weight of solution in weighing bottle.
l = length of tube in decimetres.
S = weight of substance.

Example :

1. Cane sugar.
28·025 mgm. in 5 ml. water.
According to (1) :— $c = 5{\cdot}605$; $l = 0{\cdot}5$ dm. ; $\alpha_D^{20} = +\ 1{\cdot}87°$.

$$[\alpha]_D^{20} = \left(\frac{+\ 1{\cdot}87 \times 100}{5{\cdot}605 \times 0{\cdot}5}\right) = +\ 66{\cdot}7°.$$

2. Cane sugar.
In the weighing bottle, $S = 8{\cdot}920$ mgm. ; $L = 339{\cdot}62$ mgm.
In the pyknometer, $W = 0{\cdot}2211$ gm. at 20° C. ; $W_4 = 0{\cdot}2235$ ml. ; $L^t = 0{\cdot}22541$ gm. at 20° C.

From (2) and (3) :— $d_4^{20} = \dfrac{0{\cdot}22541}{0{\cdot}22350} = 1{\cdot}10085$ gm./ml.

$$[\alpha]_D^{20} = +\ \frac{0{\cdot}88° \times 339{\cdot}62}{0{\cdot}5 \times 8{\cdot}920 \times 1{\cdot}0085} = +\ 66{\cdot}4°.$$

MOLECULAR REFRACTIVITY

Refractometer Method

The Abbé refractometer is particularly suitable. Only 1 drop is required and the refractive index can be read accurately to $\pm$ 0·0001 unit. This instrument depends on the determination of the limiting angle of total reflection in a thin layer of liquid between two prisms of greater refractive index. Readings over the range n_D 1·3000–1·7000 can be made. For illumination, daylight or an electric bulb can be used. Colour compensation is provided by two direct-vision Amici prisms for the sodium line. The refracting surfaces of the compensator, which are in a plane perpendicular to the optical axis of the viewing telescope tube, can be inclined at various angles to one another, an arrangement which serves optically as a single prism with continuously variable dispersion. If the compensator is turned so that the boundary line between light and dark in the middle of the cross wires appears quite colourless, the refractive index for the sodium line n_D can be read directly on the scale.

Procedure

The instrument is opened and tilted so that the exposed surface of the fixed prism is horizontal. A drop of liquid is then placed on this surface with a pipette or with a rounded glass rod, the moveable prism is closed on it, and the apparatus fastened and then brought horizontal again. The mirror illuminator is adjusted, and the screw of the scale is turned until the line of separation between the light and dark fields is visible; the colour compensator is now adjusted so that this line is quite colourless. It is then brought exactly to the centre of the cross wires, and n_D is read on the scale with the lens. The fourth decimal place is estimated.

FIG. 89. Precision weighing pipette. Actual size.

Water from a thermostat can be circulated around both prisms. In practice, it is usually sufficient to place the refractometer (which has a screw-in thermometer) in the balance room in which the density of the liquid is to be determined. The molecular refraction is, indeed, independent of the temperature, but care must be taken that the density and refractive index are determined at exactly the same temperature. The apparatus is calibrated with a drop of water, for which n_D is 1·333 0 at 20° C.

Density. The alcoholic specific gravity of the liquid (d), referred to water at 4° C., is determined with the precision weighing pipette shown in Fig. 89 as described on p. 208.

Calculation

$$\text{Molecular refractivity (M.R.)} = \frac{M}{d}\left\{\frac{n^2-1}{n^2+2}\right\},$$

where M = molecular weight of the substance. Tables [35] are available giving $(n^2 - 1)/(n^2 + 2)$ for values of n from 1·3000 to 1·7200.

Some important atomic refractivities for the D-line are as follows [36] :—

C	2·418	O (hydroxyl)	1·525
H	1·100	O (ether)	1·643
Cl	5·967	O (carbonyl)	2·211
Br	8·856	N (prim. amino)	2·322
I	13·900	N (sec. amino)	2·502
Double bond	1·733	N (tert. amino)	2·840
Triple bond	2·398	N (nitrile)	3·118

Example :—

Benzene. $M = 78·05$; $n_D^{20} = 1·5009$; $d_4^{20} = 0·879$.
M.R., calculated = 6C + 6H + 3 double bonds = 26·31.
M.R., found = 26·16.

Other Methods

The Immersion Method is based on the immersion of a solid in each of a series of liquids of known refractive indices, until one is found in which it is no longer visible ; the refractive index of the solid is then the same as that of the liquid. The converse procedure is applied to the determination of the refractive index of a liquid. The accuracy of this method is, however, not so great as that of the refractometer method (sensitivity, $\pm$ 0·003), and the only advantage it has is that expensive apparatus is not required.

The Refractometer Method has been made more convenient recently by using one standard liquid at varying temperatures to produce a known range of standard refractive indices, in place of a number of standard liquids at the same temperature. In an improved method for solids, Jelley [37] and Frediani [38] place a crystal in a modification of the Abbé refractometer, and then raise the temperature of the prisms electrically until the melting-point is reached ; the sensitiveness is $\pm$ 0·002, but the method cannot be used for substances which volatilise or decompose at or near their melting-points. The method is also used for identification purposes,[39] and for this purpose Frediani describes a modification of the Fischer refractometer, which makes possible the simultaneous determinations of melting-points up to 200° C. and refractive indices between 1·300 and 1·900 ; the respective errors are 1°–2° C. and $\pm$ 0·002 unit.

Other methods are also described by Alber and Bryant [40] and by Kirk and Gibson.[41]

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CHAPTER VI

MISCELLANEOUS PHYSICAL METHODS

THE methods dealt with in this chapter belong rightly to the sphere of physics, or of physical or inorganic chemistry. However, since they frequently play important *rôles* in organic analysis, it is felt advisable that they should be included. For the above reasons they are not dealt with in the same detail as are the other and purely organic methods described in this book. The detail given is, in fact, roughly proportional to the importance of the subject from the point of view of organic chemistry, but in all cases sufficient references to recent advances are provided to enable those interested to obtain further information. It may be noted that most of the methods described are micro-modifications of the ordinary macro-methods.

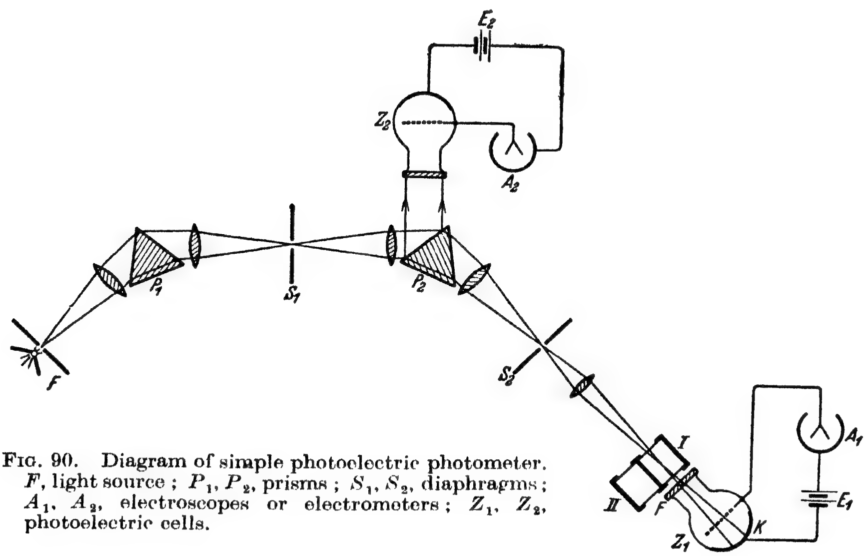

FIG. 90. Diagram of simple photoelectric photometer. F, light source ; P_1, P_2, prisms ; S_1, S_2, diaphragms ; A_1, A_2, electroscopes or electrometers ; Z_1, Z_2, photoelectric cells.

Absorption Spectroscopy

Principles

Inside an evacuated glass blub (Z_1, Fig. 90) is an alkali metal surface, which is charged negatively by a battery E_1. If a beam of light falls on this surface, electrons are liberated, and are conducted to a second electrode by means of the induced E.M.F., produced by the electric field ; from there, they pass to a sensitive fibre-electrometer A_1, the case of which is connected with the positive pole of the battery E_1. The photoelectric current which results from the exposure can therefore, be measured by the rate of charging of the electrometer ; the progress of charging is shown by the travel of the electrometer pointer along a scale. The number of electrons split off, and therefore the strength of

the photoelectric current (expressed by the rate of charging of the electrometer), is directly proportional to the intensity of the light falling on the photo-cell.

If the solution to be examined is placed in front of the photoelectric cell, in the path of the light, rays of certain wave-lengths are absorbed during their passage through the solution, in a way characteristic of the molecules in it. The measurement of intensity of the light passing through is always carried out in comparison with the solvent used, so that effects due to the latter are eliminated. The changes in intensity due to the dissolved molecules are then directly proportional to the reading on the calibrated electrometer.

The intensities of the light passing through the solution and the solvent enable the so-called molecular absorption constant (x) to be calculated from the Lambert-Beer Law, which is the basis of the whole of absorption photometry.

This law is expressed by the following formula :—

$$I = I_0 \, . \, e^{-x.d}$$

I_0 is the intensity of the light falling on a layer of thickness d ; I the intensity of the light emerging from it ; x the molecular absorption constant, *i.e.*, the absorption constant for 1 gm.-molecule per litre of the substance concerned. Conversely, x is the reciprocal of the layer-thickness, which for unit concentration, reduces the intensity of the incident light of a definite wave-length to one-tenth of the original. The equation may also be written :—

$$x = \frac{1}{c.d} \log_e \frac{I_0}{I} = \frac{2{\cdot}3}{c.d} \log_{10} \frac{I_0}{I}$$

The electrometer readings obtained on alternately inserting the pure solvent and the solution, give the intensities I and I_0 directly. The thickness of the layer (d) is known from the dimensions of the absorption vessel. The concentration c is calculated from the weight of substance taken. The molecular absorption constant may thus be found for rays of different wave-lengths, and the values plotted so that the abscissæ show wave-lengths in $m\mu$, and the ordinates x in cm.^{-1} (Fig. 91). The curve so obtained for a wide range of wave-lengths is characteristic of the substance concerned.

Apparatus

Full details of the technique of absorption spectrophotometry and a review of the uses of the different methods, is given by Twyman and Allsopp.[1]

Pohl's spectrophotometer [2] consists of a Lehmann-Rudert double monochromator and a photoelectric photometer ; two models are available, namely, for measurements in ultra-violet light and in the visible spectrum ; the former is shown in Fig. 90.

The rock salt prism P_1 and the quartz prism P_2 are each mounted on a table which enables automatic adjustment to the position of

minimum deviation to be obtained; the prism-tables are connected by a three-sided iron support, and each has also a second support which can be displaced horizontally (not shown in the illustration) on which the other parts are mounted. The source of light (F) is a mercury-quartz lamp (for the long-wave ultra-violet region) or the spark spectrum of cadmium, gold, aluminium or magnesium (for the short-wave ultra-violet region), and it is contained in a box provided with a slit. Monochromatic light is obtained by means of the "double-monochromator" embodied in the prisms, and by the diaphragms S_1 and S_2. With the aid of a lens, the image of the slit S_2 is focused on the window of the cell. The measuring light thus passes through the vessels I (solution) or II (solvent), into Z_1 which is in circuit with the electroscope A_1.

Variations in the light absorption in the cells are shown by the movement of the electroscope. Fluctuations in intensity of the source of light during the measurement are eliminated by projection of the small proportion of the light reflected at the surface of the prism P_2 into a second photocell Z_2, which is also connected with an electroscope (A_2); the reading on this electroscope is then compared with that on A_1. Both electroscopes are calibrated, and one scale-division corresponds with 0·05–0·10 volt between leaf and case. If this relationship is not linear, a calibration curve should be plotted. A suitable type of electrometer may be used instead of an electroscope at A_1 and A_2.

The apparatus for measuring absorption in the visible spectrum differs from that described above in that the three-sided bars are all fixed, and that there is no second photoelectric photometer. The prisms are of flint glass, and a Nernst lamp is the source of light; its steadiness renders a second photometer superfluous. The individual lines of the spectrum at intervals of 5 mμ are obtained by displacement of the slit S_1, which can travel along a calibrated micrometer scale. A metronome is used for timing the rate of charging of the electroscope.

Procedure

The measurement of the absorption spectrum really belongs to the sphere of the physicist. Once, however, the apparatus is set up and tested, the method can be mastered with practice. The very sensitive electroscope or electrometer must be treated with special care.

Usually 1·5–2·0 mgm. of the pure substance are weighed out on the micro-balance, into a 25-ml. measuring flask, from a weighing tube with a handle. Sufficient solvent is added to exceed the solubility ratio of the substance, and after mixing well, all of the latter should have dissolved. If only a very small amount of material is available the same concentration can naturally be obtained, *e.g.*, by dissolving 0·5 mgm. in 10 ml. The weight is also adjusted according to the absorptive power of the substance concerned. This can be ascertained by placing the cell containing the solution immediately in front of a

small plate of uranium glass (which is held just in front of the slit S_1 in the path of the rays), and noting whether the rays in question still pass through. If at the given concentration these pass through only very weakly, or not at all, a lower concentration is used. In order that, for example, the absorption band of the longest wave-length (443 mμ) of azobenzene may be measured, about 12 mgm. of the substance must be dissolved in 20 ml. of alcohol. If the concentration selected is too high (so that all light is absorbed) dilution with the solvent only is necessary; any measurement obtained with the original concentration is then repeated. All the results may then be converted in terms of the new concentration.

For measurement in ultra-violet light the solution in the measuring flask is poured into a quartz cell *I* of known thickness (in one case, 0·114 cm.); a double seal avoids oxidation of sensitive substances by the air in ultra-violet light. Pure solvent is placed in a second quartz cell *II* of the same dimensions. Both absorption vessels are placed immediately in front of the photocell Z_1, on a sliding arrangement which permits *I* and *II* alternately to be inserted in the beam of light. To avoid reflection, the cell must be exactly normal to the beam of light. Some wave-lengths of important lines in the ultra-violet region are given in Table I.

The room is now darkened, the mercury lamp or other source of light is turned on, and the lens between P_1 and S_1 is adjusted to ensure the sharpness of the separate lines of the spectrum on a small plate of uranium glass which is held obliquely in front of the slit S_1. It is usual to begin with the mercury line 365 mμ, and to make sure that the light falls normally on the lens and not obliquely. Displacement of the photocell, and adjustment of the lens, enable a sharp image of the lines of the spectrum to be obtained in the plane of the slit S_2. The cell containing the solvent (*II*) is now moved into the beam of

Table I. Wave-lengths of the More Important Ultra-violet Lines

Wave-length mμ	Metal	Wave-length mμ	Metal	Wave-length mμ	Metal	Wave-length mμ	Metal
365	Hg	296	Hg	248	Hg	214	Cd
360	Cd	289	Hg	240	Hg	211	Au
346	Cd	280	Hg	238	Hg	208	Au
340	Cd	275	Hg	234	Hg	204	Au
334	Hg	270	Hg	232	Cd	200	Au
326	Cd	265	Hg	230	Hg	199	Al
313	Hg	257	Hg	226	Cd	193	Al
309	Mg	254	Cd	219	Cd	186	Al
302	Hg						

light. The slits S_1 and S_2 are set according to the intensity of the line used, as previously ascertained from control tests with the electroscope.

The actual measurement now begins. The photographic shutter behind the slit S_1 is closed (unless a spark spectrum is used) and both electroscopes are earthed. If the leaves remain in contact no disturbances are present. The shutter is now opened, or the spark spectrum is inserted, and as soon as the electroscope A_2 indicates a definite reading (*e.g.*, 20 scale-divisions) the rays are cut off and the other electroscope A_1 is read; the reading on A_1 gives I_0 directly. The cell with the solution (I) is now inserted and a similar determination is made to find I. If the reading increases in proportion to the voltage, the ratio I_0/I may be found directly; otherwise, the corresponding voltages must be deduced from the calibration curve.

In this way the measurements are extended towards the shorter wave-lengths until the solvent used no longer permits the passage of light, *i.e.*, specific absorption by the solvent occurs. So far as possible, the solvents used are those which absorb only rays of very short wave-lengths, *e.g.*, hexane, alcohol or water.

The visible spectrum range is used if an absorption of the solution in this range is anticipated by reason of its ordinary colour; or, better, from the results of the measurements in ultra-violet light. For this, the absorption vessel is placed in front of the photocell Z_1 as before, the Nernst lamp is switched on, and the slit S_1 is adjusted with the help of the measuring-drum (according to the calibration table) for the passage of rays of definite wave-length, beginning at 370 mμ. The cell containing the solvent is now brought in front of the photoelectric cell, the metronome is started, the photographic shutter is opened, and the travel of the electroscope during a definite interval of time (as indicated by the metronome) is noted; the light is then switched off and the scale divisions I_0 read, and I is obtained similarly. The slit S_1 is adjusted by the micrometer drum for the next wave-length, which is 5 mμ higher, and I_0 and I are read similarly for this. This procedure is continued by increasing the wave-length by 5 mμ each time, until the solution no longer absorbs the rays used. The molecular absorption constants for the individual wave-lengths are then calculated from the ratios I_0/I.

Calculation and Example

In an actual experiment,[3] 0·855 mgm. of purified lactoflavin (molecular weight, 376) was weighed into a 10-ml. measuring-flask, which was filled to the mark with water in which the substance dissolved completely on mixing well. The concentration,

$$c = \frac{0{\cdot}855 \times 100}{1000 \times 376} = 2{\cdot}27 \times 10^{-4} \text{ gm. molecule per litre.}$$

The thickness of the layer, $d = 0{\cdot}114$ cm.

$$\text{The factor, } f = \frac{1}{c} = \frac{1}{2{\cdot}27 \times 10^{-4}} = 4{\cdot}4 \times 10^{3}.$$

According to the equation on p. 215,

$$x = \left(\frac{4{\cdot}4 \times 2{\cdot}3}{0{\cdot}114}\right)\left(\log \frac{I_0}{I}\right)10^3.$$

The first term of the equation holds for all the measurements of the same kind on the same apparatus, and only the logarithms of the intensity ratios for the different wave-lengths are required for the calculation.

Table 2

Wave-length $m\mu$	I_0 (Scale-divisions)	I (Scale-divisions)	I_0 (Scale-divisions)	$\log \frac{I_0}{I}$	$x/10^3$
510	33·1	32·4	33·1	0·009	0·8
500	32·9	30·0	32·9	0·040	3·44
490	32·0	26·0	32·0	0·090	7·7
480	32·3	21·1	32·3	0·185	16·0
475	33·9	20·1	33·9	0·227	19·5
470	30·9	17·0	30·9	0·260	22·4
465	29·1	15·1	29·1	0·285	24·4
460	33·0	16·9	33·0	0·290	25·0
455	33·1	15·8	33·1	0·322	27·6
450	30·1	14·1	30·0	0·330	28·4
445	32·9	15·1	32·9	0·338	30·0
440	28·1	13·0	28·1	0·334	29·6
435	30·1	14·0	30·0	0·331	29·4
430	21·0	10·5	21·0	0·301	26·7
425	32·0	17·0	32·0	0·274	23·5
420	33·0	18·0	33·0	0·263	22·6
415	30·9	17·8	31·0	0·239	20·5
410	30·0	18·1	30·0	0·220	18·9
400	30·7	20·0	30·8	0·188	16·2
390	34·9	21·2	34·9	0·216	18·5
380	18·1	10·0	18·1	0·257	22·0
375	9·0	5·0	9·0	0·255	21·9
370	4·0	2·3	4·0	0·243	20·9
365	33·0	24·0	33·0	0·158	18·75
360	30·7	19·6	31·0	0·216	18·6
346	31·3	20·4	31·3	0·190	16·4
340	30·8	21·6	30·9	0·168	14·5
334	30·3	22·1	30·3	0·147	12·7
326	29·9	23·8	29·9	0·109	9·4
313	30·0	27·2	30·0	0·050	4·32
302	30·0	28·0	30·0	0·035	3·0
289	30·7	20·4	30·6	0·188	16·2
280	31·8	11·0	31·8	0·467	40·0
275	30·3	6·9	30·3	0·654	56·4
270	32·0	5·0	32·0	0·814	69·9
265	29·0	4·0	29·0	0·844	72·5
254	31·1	7·3	31·0	0·635	54·8
248	31·0	12·3	31·0	0·408	35·0

In Table 2, *e.g.*, for $\lambda = 510\ m\mu$, $x = 0{\cdot}8 \times 10^3$. The corresponding

values for λ and x are shown on the graph (Fig. 91). This lactoflavin absorption curve has three marked maxima, viz., at 266, 378 and 445 mμ, and at respective heights of 73, 22 and 30 mm. The absorption band at 445 mμ (which is in the visible spectrum) corresponds with the yellow colour of the substance, and the height of the maximum is a criterion of purity. Measurement of the absorption of this single line, therefore, is a check on the purity of the substance, so long as the impurities present do not accidentally occur in the same region of absorption. Such measurements are obtainable with very small expenditure of substance and time, in marked contrast with an ordinary analysis or biological test, especially as the melting-point is in this case not sufficiently sensitive for checking completely the purity of the natural product.

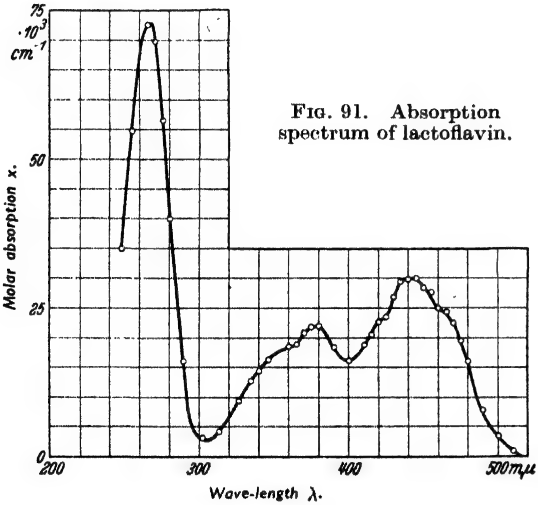

FIG. 91. Absorption spectrum of lactoflavin.

Notes

The importance of the method is thus its great accuracy and sensitivity. Well-marked maxima can be determined to within $\lambda \pm 1$ mμ, and the limiting error for the height of the band is about $\pm 2\%$. Even amounts of impurities which can scarcely be detected by other physical properties (*e.g.*, the melting-point) may visibly influence the heights of the bands.

It must, however, always be borne in mind that interpretations of the absorption spectra are based in great measure on analogies drawn from the spectra of a large number of comparison materials ; they are thus purely empirical, and it is not yet possible to deduce accurately the absorption behaviour of chemical compounds from their structural formulæ in the same way as molecular refractivity, for example, may be calculated. The work of Hausser and his colleagues [4] on the relationship between the light absorption and the double-linkages of homologous series is, however, a very promising beginning. In many cases, difficulties may arise owing to influences of the solvent which have not as yet been fully investigated. Also many cases occur, where, in spite of constitutional differences between two compounds, the absorption spectra are almost identical. The method must thus be used in conjunction with other physico-chemical and purely chemical methods for the determination of the constitution. Its uses in colorimetry are referred to on p. 221, and recent advances are reviewed by Müller.[5]

Surface Tension and Viscosity

Surface Tension. The capillary rise method [6] is simple and accurate (error, ± 0·5%). The apparatus shown in Fig. 92 requires only 0·1 ml. of sample. The capillary portion has a diameter of 0·2 mm., and the density is given by the formula $HDgRR'/2(R—R')$, where H is the height of the column (when this has become constant), D the density of the liquid (see p. 190), g the gravity acceleration constant, and R

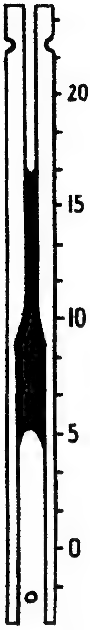

FIG. 92. Micro-determination of surface tension.

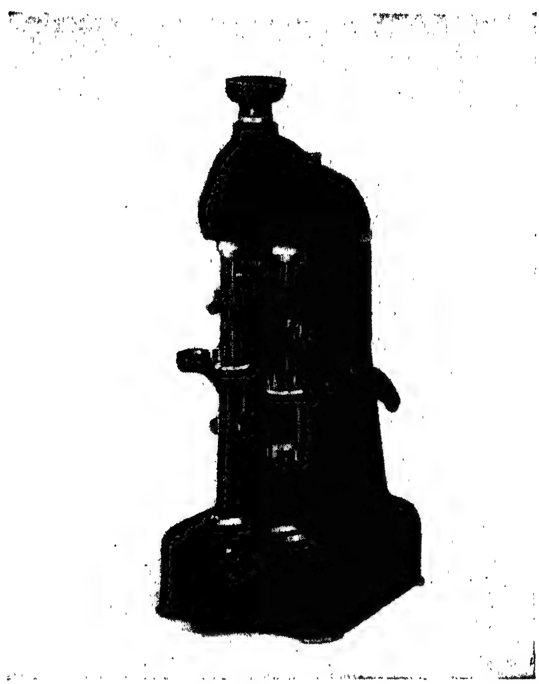

FIG. 93. Micro-colorimeter.

and R' the radii of the wide and narrow portions of the apparatus, respectively.[7]

Variations of this method are described by Bowden,[8] by Sugden [9] and by Ferguson and Kennedy.[10]

Viscosity. The macro-type of instrument with suitable modifications is used. It has certain inherent errors which, however, have been overcome (partly, at any rate) by the improvements suggested by Bowman [11] and by Cannon and Fenske.[12] The original papers should be consulted for full details.

Colorimetry

This method of quantitative analysis is of more importance in inorganic than in organic chemistry. There are, however, certain applications [13-15] (*e.g.*, determinations of proteins and metals, *cf.* p. 126)

which justify a short reference to it here. A very simple micro-method can be devised by using thin, flat-bottomed test-tubes about 5 mm. in diameter and 20 mm. high, and comparing coloured solutions just as in the Nessler method. Micro-modifications of the more eleborate Duboscq type colorimeter also exist, and an illustration of one of these is shown in Fig. 93 ; the cups have a capacity of 1 ml. and provide for a depth of fluid of 20 mm. Photoelectric spectrophotometers and other instruments are also used for evaluating colours numerically in connexion with colorimetric analysis ; an example is cited above. The method is, however, unnecessarily elaborate for most purposes.

Nephelometry [17]

Nephelometric methods, in which turbidities are matched or measured instead of colours, play a similar part in organic analysis. Thus, Davis and Parke [18] have measured the solubilities of polycyclic hydrocarbons with an error of 5–10% by adding water, and determining the concentration of hydrocarbon beyond which further dilution produces no reduction in the degree of light scattering. The limit of solubility which can be measured in this way is 1 μgm. per litre. An example of a different nature is the determination of small amounts of nicotine (*e.g.*, 0·2 mgm.) in a micro-nephelometer [19] ; the reagent is a solution of silicomolybdic acid, and the error is about 2%. See also p. 112.

Gas Analysis

Those micro-gas analyses which are incidental to the methods described elsewhere in this book are dealt with under the appropriate headings (see especially pp. 76, and 142). In particular, the micro-Dumas' method for the determination of nitrogen described on pp. 63 *et seq.* gives a good indication of the special technique involved in work of this kind. Many of the commoner methods of gas analysis are concerned with inorganic rather than with organic analysis, but certain recent developments are common to both spheres, and are worthy of special mention.

The Constant Volume Method has been adapted by Ambler [20] for micro-purposes, and it enables 1-ml. samples of gas to be analysed with an error of only about 1%. It is based on the measurement of the pressure of a gas when it occupies a certain volume at a definite temperature, as distinct from the more usual method in which the volume of the gas is measured at a constant pressure and temperature. In addition, rubber connexions, which may cause leaks or air-locks, are eliminated ; the volume of absorbing solution is reduced so as to minimise its solvent effect, as distinct from its specific chemical absorption for the gas ; and the sensitiveness of the reading devices is increased. Levelling and parallax difficulties are avoided, and only small quantities of mercury and reagent are required. With a little practice the method is both simple and rapid, and mixtures of nine constituent gases have been analysed in 30 mins.

The Apparatus (Fig. 94)

This consists essentially of three vertically connected glass bulbs, 1, 3 and 6 ml., respectively, in volume, each or all of which are used, according to the volume of sample available. They are enclosed in a constant-temperature water-jacket, and are connected at the base with the manometer M_1, and at the top, through the three-way taps T_1 and T_2, either to the air (at A), to a reservoir (R_3), or to a 25-ml. absorption bulb B which, in the model illustrated, is also provided with explosion electrodes. The connecting tubes are glass capillaries (bore, 1 mm.). The other tubes shown on the manometer stand are included for convenience. M_2 is an auxiliary manometer which enables the progress of absorption to be followed, and N can be nsed as a barometer tube.

The manometer reading (k) when the bulbs are filled at atmospheric pressure must be known, but need be determined only once, namely, by opening the bulbs to the atmosphere *via* T_1, T_2 and R_3, and bringing the mercury in them to the appropriate mark by means of R_1.

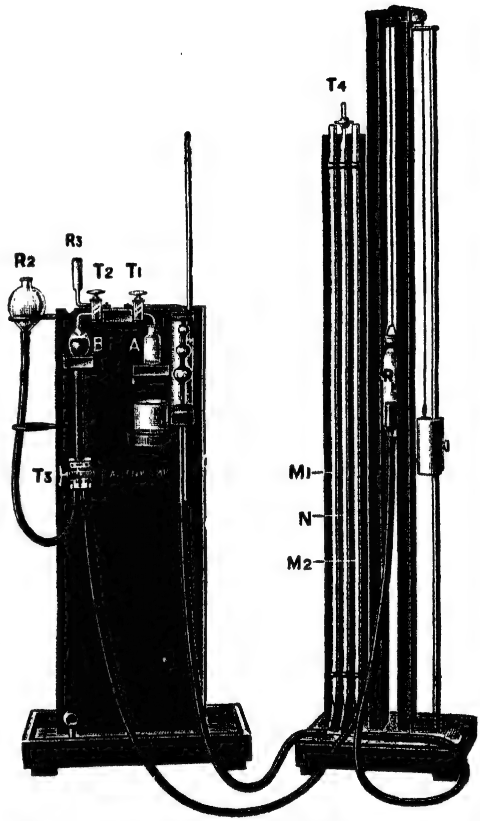

FIG. 94. Ambler apparatus for the microanalysis of gases.

Procedure

Fill B with mercury, and transfer the sample to it through A by lowering the reservoir R_2; if the sample is contained in an inverted test-tube, a capillary U-tube may be attached to A for this purpose. Manipulate T_1 and T_2 so that the gas is transferred to one or more of the bulbs. Measure the volume by taking the manometer reading when the bulb system is filled with gas to the 1-, 3- or 6-ml. mark, as required; during this process the top of the bulbs may be sealed against leaks by allowing mercury from R_3 to fill the horizontal tube between T_1 and the top bulb, *via* T_2 and T_1.

Now introduce into B, *via* A, the necessary quantity of gas-absorbing solution, transfer the gas from the bulbs to B for absorption (which is aided by shaking this portion of the apparatus *en bloc*), and

pass the residue back to the bulbs for measurement, taking care that the sealing column of mercury is at the same point as in the original measurement, and that the temperature of the water-jacket has not changed.

Calculation and Notes

Let a cm. = manometer reading before absorption.
b cm. = manometer reading after absorption.
k cm. = manometer reading with bulbs at atmospheric pressure.
c cm. = barometric pressure.
w cm. = vapour-pressure of water at the temperature of the determination (see p. 145).
v ml. = volume of bulb system used.

Then, the partial pressure of the gas being analysed = $(a + c - k - w)$; this need, of course, be determined only once for each analysis. Then the volume of the gas at normal pressure is, initially,

$$v(a + c - k - w)/76 \text{ ml.}$$

The corresponding volume after absorption is,

$$v(b + c - k - w)/76 \text{ ml.},$$

so that the volume of gas absorbed is $v(a - b)/76$ ml.; or,

$$100\,(a - b)/(a + c - k - w)\%.$$

Haden and Luttropp,[21] and Spence,[22] have used this method in conjunction with high-vacuum and refrigeration technique for the analysis of hydrocarbon mixtures; indeed, the latter type of technique has itself proved of considerable use in problems of this nature. Thus, Sebastian and Howard [23] used the characteristic form of the temperature-vapour pressure curve obtained from a 1-ml. sample for the determination of the constituents of hydrocarbon mixtures; the method is based on the fact that for each hydrocarbon the change of pressure with temperature is relatively rapid over a certain temperature range. A disadvantage of the method is that it must be calibrated against known mixtures, but an advantage is that the sample can be recovered eventually unchanged. In a similar method Euchen and Knick [24] absorb the gases on active carbon in a U-tube at about − 103° C.; this is then warmed gradually to 250° C. The resulting pressures in the tube and in the collecting vessel are plotted against the time; the former show the progress of the fractional desorption, and the latter enable the quantities evolved to be calculated. A more elaborate variation of this method due to Küchler and Weller [25] enables mixtures of unsaturated and saturated hydrocarbons to be analysed.

With certain gas mixtures the method of Price and Woods [38] is more rapid and sufficiently accurate, and it is suitable for 0·004 cb. mm. of sample. Briefly, the diameter of the gas-bubble sample is measured

under a microscope ; the bubble is then brought into contact successively with appropriate chemical absorbents, and its diameter is measured again after each treatment to obtain the volume of gas removed.

Other Physical Methods

Electrometric Methods

Such methods really fall under the heading of inorganic analysis and must be dismissed here very briefly, although reference may be made to the micro-electrodeposition of copper (p. 123) and to the use of potentiometric titration as an aid to certain of the other methods described in this book. An example of the latter nature is the titration, with a specially designed glass electrode, of the solution obtained following a Kjeldahl digestion (Borsook and Dubnoff) [26] ; the error of this method is $\pm$ 1%.

Polarography [27, 28]

This method involves expensive apparatus, but it has given very useful results in the determination not only of inorganic ions, but also of small traces of certain oxidisable and reducible organic substances (*e.g.*, vitamins). The solution to be analysed is placed in a vessel on the bottom of which is a layer of mercury, the anode. The cathode is a stream of fine drops of mercury, falling from a reservoir through the solution. A difference in potential between the electrodes is applied by means of a special potentiometer, which increases this difference automatically as the analysis proceeds. A potential difference-current graph is traced automatically on a rotating drum. Provided the applied potential does not exceed the decomposition potential of any of the salts in solution, there will be no current. When the first decomposition-potential is reached, a current proportional to the concentration of the ions in the solution will flow. When the decomposition-potential of a second compound is reached, this current will increase, and so on. A stepped curve is thus obtained, the height of the steps on the current axis being proportional to the concentration of the ions. Several elements can be determined simultaneously and rapidly. For example, the copper, zinc and lead in a brass can be found in 15 mins., provided that the apparatus has been standardised by the use of a similar alloy of known composition.

Not only will the apparatus determine the concentration of metallic ions and of other ionisable and reducible compounds, but it will also determine any substance which can be adsorbed on mercury and reduced. Thus, the instrument is of use in metallurgical analysis and in the examination of water, gas, sugar, beer, dye-liquors, etc. ; 0·01-0·05% of nitrobenzene in aniline has also been determined in this way.[39] Special cells for micro-work (capacity, less than 0·5 ml.) are now available.[29]

Radioactive Indicators and Tracer Isotopes [30]

In this method substances are "labelled" by addition of small amounts of other substances (*e.g.*, radioactive isotopes), which are easily detectable (*e.g.*, by an electroscope), but which cannot be separated from them by the ordinary methods of chemical analysis. Thus, stable isotopes have been used in the analysis of mixtures of organic substances which are closely related chemically, a good example being the analysis of amino acids (Rittenberg and Foster [31]). However, the only isotopes commonly available for the purpose are deuterium and N_{15}, although radioactive phosphorus (P_{32}), which is chemically indistinguishable from P_{31}, has been used in the study of phosphorus metabolism.[40] The method has wider applications in inorganic microanalysis.

Fluorescence Analysis in Ultra-Violet Light

Most of the better-known applications of this method apply to inorganic and technical microanalysis, but examples of its uses in quantitative organic microchemical analysis are given by Radley and Grant [32] and by Haitinger [33]; noteworthy among these are determinations of vitamin B_1.[36, 37] The principle of the method is the production of fluorescence effects which are usually invisible in ordinary visible light, but which can be seen under an ultra-violet lamp from which visible light is excluded by means of a special filter. The only special apparatus required is the ultra-violet lamp with filter. Since these fluorescence effects can often be produced by extremely small quantities of certain substances, the methods in which they are used sometimes have high degrees of both sensitivity and selectivity. As an example, certain substances which become fluorescent or non-fluorescent at a volumetric end-point may be used as indicators; they often enable very dilute solutions to be titrated, and they can function even in solutions which are normally so coloured or turbid that the change in colour of an ordinary indicator is invisible. Colorimetric and volumetric methods involving the formation of a fluorescent reaction-product have also been developed, *e.g.*, the determination of quinine as the sulphate (Grant,[34] and Nicholls [35]).

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CALCULATION OF THE RESULTS OF MICROANALYSES

This is always carried out logarithmetrically using exact values for the atomic weights and preferably, logarithmic tables for chemists. The percentage of all the elements in the formula of a compound should be calculated, even though only one or two of them are required ; thus, the percentage of oxygen should always be calculated. The accuracy of the calculations can then be checked, by ascertaining that the sum of the percentages is 100. The necessary factors for the calculation of the analyses, and their logarithmic mantissæ are given below in tabular form. In addition, factors for the determination of methoxyl, ethoxyl, methyl and carboxyl groups are also given here.

Gravimetric Factors

Required	Found	Factor	Log Factor
C	CO_2	0·2727	43573
H	H_2O	0·1119	04875
Cl	AgCl	0·2474	39334
Br	AgBr	0·4255	62894
I	AgI	0·5406	73284
S	$BaSO_4$	0·1373	13782
P	$MgNH_4PO_4 . 6H_2O$	0·1264	10174
P	$Mg_2P_2O_7$	0·2786	44499
P	$(NH_4)_3PO_4 . 12MoO_3$	0·016524	21811
P_2O_5	$(NH_4)_3PO_4 . 12MoO_3$	0·03783	57784
As	$Mg_2As_2O_7$	0·4827	68371
$O.CH_3$	AgI	0·1321	12096
$O.C_2H_5$	AgI	0·1918	28287
CH_3	AgI	0·06398	80604
C_2H_5	AgI	0·12380	09273

Table for Determinations of Metals in Organic Salts

Required	Found	Factor	Log Factor
Silver	Ag	—	—
Aluminium	Al_2O_3	0·5291	72357
Gold	Au	—	—
Barium	$BaSO_4$	0·5885	76972
Bismuth	Bi_2O_3	0·8965	95269
Calcium	$CaSO_4$	0·2944	46889
Cadmium	$CdSO_4$	0·5392	73176
Cobalt	Co	—	—
Chromium	Cr_2O_3	0·6843	83522
Copper	CuO	0·7989	90250
Iron	Fe_2O_3	0·6994	84473
Potassium	K_2SO_4	0·4488	65203
Lithium	Li_2SO_4	0·1263	10133
Magnesium	MgO	0·6032	78044
	$MgSO_4$	0·2020	30541
Manganese	$MnSO_4$	0·3638	56086
Sodium	Na_2SO_4	0·3238	51026
Nickel	Ni	—	—
Lead	$PbSO_4$	0·6833	83458
Platinum	Pt	—	—
Silicon	SiO_2	0·4692	67150
Tin	SnO_2	0·7881	89654

Volumetric Factors

Required	ml. of Standard Solution used	Factor	Log Factor
As	N/100–$Na_2S_2O_3$	0·3748	57380
Br	N/100–NaOH	0·7992	90266
Cl	N/100–NaOH	0·3546	54974
I	N/50–$Na_2S_2O_3$	0·4231	62644
N	N/100–HCl	0·1401	14638
S	N/100–NaOH	0·1603	20493
	N/50–NaOH	0·3206	50596
CH_3	N/50–$Na_2S_2O_3$	0·3005	47780
C_2H_5	N/50–$Na_2S_2O_3$	0·5808	76403
CH_3(C)	N/100–NaOH	0·15023	17676
C_3H_6 =	N/20–Iodine	0·3505	54469
— O.CH_3	N/50–$Na_2S_2O_3$	0·10341	01458
— O.C_2H_5	N/50–$Na_2S_2O_3$	0·15013	17647
COOH	N/100–NaOH	0·45008	65323
CH_3.CO—	N/100–NaOH	0·4302	63370
C_5H_6.CO—	N/100–NaOH	1·0504	02135
CH_3.COOH	N/100–NaOH	0·60031	77838

Multiples of Organic Groups

$O.CH_3$	Log	$O.C_2H_5$	Log
1. 31.02 (34)	49169	1. 45·04	65360
2. 62·05	79273	2. 90·08	95463
3. 93·07	96881	3. 135·12	13062
4. 124·10	09376	4. 180·16	25568
5. 155·12	19067	5. 225·20	35257
6. 186·14	26985	6. 270·24	43175

CH_3	Log	COOH	Log
1. 15·02 (34)	17677	1. 45·008	65329
2. 30·05	47781	2. 90·016	95431
3. 45·07	65391	3. 135·024	13039
4. 60·10	77884	4. 180·032	25534
5. 75·12	87576	5. 225·040	35226
6. 90·14	95494	6. 270·048	43144

AUTHOR INDEX

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Principal References in Heavy Type

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LONDON AND TONBRIDGE